Establishing *Macrophomina*-infested Research Plots in California's Central Coast

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Summary

Crown rot, caused by *Macrophomina phaseolina*, is an emerging disease in California strawberries since the phase-out of methyl bromide fumigation. In order to study the disease under field conditions, *Macrophomina*-infested field sites are needed. Ideally, field sites should be located in an area conducive to disease but isolated from the major production regions with strict sanitation measures to prevent the escape of inoculum or introduction of non-target pathogens. A one-acre *Macrophomina*-infested field was established at Cal Poly San Luis Obispo using locally collected isolates of the fungus. The site is 30 miles from the nearest production in Santa Maria and has slightly higher temperatures and heavier soil (silty clay). Three inoculation techniques (i.e., dip, cornmeal-sand and corncob) were evaluated on the cultivars 'Albion', 'San Andreas' and 'Strawberry Festival'. Differences in the rate of plant collapse were recorded, with dip-inoculated plants collapsing as early as 30 days after inoculation, followed by cornmeal-sand and then corncob inoculation methods. All cultivars were susceptible to the pathogen; with 'Strawberry Festival' most susceptible, followed by 'Albion' and then 'San Andreas'. All cultivars and inoculation techniques in this study could be used in future experiments depending on the objectives. For immediate purposes, the cornmeal-sand inoculum provides the optimum rate of disease progress (not too fast or too slow) to evaluate strawberry genotypes for their ability to resist crown rot under field conditions. We will be

evaluating numerous cultivars and elite selections in this field using the cornmeal-sand inoculation technique during the 2016-2017 production season.

No information is provided in this report regarding the second field location at Monterey Bay Academy in Watsonville.

Introduction

One of the negative consequences of the phase-out of fumigation with methyl bromide is the reemergence of old and emergence of new soilborne diseases. Crown rot caused by *M. phaseolina* is a new disease that has emerged in strawberry production areas worldwide as methyl bromide use has decreased. The disease is favored by high temperatures and plant stress caused by factors such as high fruit load, drought, poor plant nutrition and salinity. The pathogen forms melanized microsclerotia that can survive in the soil for many years.

Practices such as crop rotation, anaerobic soil disinfestation (ASD), and fumigants such as chloropicrin and allyl-isothiocyanate (AITC) provide only marginal control of crown rot. Host plant resistance holds one of the most promising avenues for controlling this disease and field sites are needed in order to evaluate strawberry germplasm for the ability to grow and set fruit in the presence of the pathogen. Moreover, a number of chemical and alternative treatments are available for use, but lack credible evaluation under appropriate conditions.

Commercial field sites with a history of the disease are difficult to utilize for research. Growers are understandably concerned about preserving high levels of inoculum in untreated areas for the sake of research. This poses a risk to commercial fruit production in infested fields as there is potential for pathogen spread to other fields. Ideally, research should be conducted in infested fields that are isolated from production regions and conducive to the disease. The objective of this work was to establish a *Macrophomina*-infested field on the campus of Cal Poly San Luis Obispo in order to conduct studies on management of the disease in a field setting.

Materials & Methods

A one-acre field site was selected on the Cal Poly San Luis Obispo campus at Field 35b, located on the corner of Mt. Bishop Road and Technology Park (35° 18'20.21" N; 120° 40'23.39"). This field has been used for the last 20+ years to grow field crops such as corn, alfalfa and triticale for the Cal Poly dairy. It has also been used as a dairy lagoon discharge site; strawberries have never been grown in this field. Soil type is a Salinas silty clay loam with a gentle downward slope to the south.

Because of the unknown soilborne disease history and the need to start the experiment with soil as pathogen-free as possible, this field was fumigated with Tri-Con 50/50 (50% methyl bromide/ 50% chloropicrin) at 350 lb/acre on May 23 and planted on July 6 and 7, 2015. A summer planting was selected since crown rot is favored by high temperatures.

Bare root transplants were set into beds on 64-inch centers, with four rows of plants per bed and 16-inch spacing between plants. Two lines of drip tape were used per bed. The total plot size was 1.0 acre consisting of 47 beds x 150 ft long, including three non-planted buffer beds used to separate 11 non-infested beds from 33 pathogen-infested beds (Figure 1). Each bed had 488 plants (122 plants per row/plant line).

Throughout the experiment, strict sanitation measures were followed 1) to prevent movement of soil from infested to non-infested sides of the field, and 2) to prevent the introduction of soil from other fields. This includes an 8-foot tall fence surrounding the field, locked gates on both ends, signage informing the public "DO NOT ENTER", plastic shoe covers for field workers and routine use of phenol-based surface disinfectants on shoes and equipment entering and leaving the field.

Soil or plants were inoculated with multiple isolates of *M. phaseolina* using different methods and plants were then monitored for disease symptoms on a regular schedule. Disease progressed over nearly 14 months before the plants were disked in on September 1, 2016. Three inoculation techniques (dip, cornmeal-sand and corncob granules) were evaluated on the cultivars 'Albion', 'San Andreas' and 'Strawberry Festival'. These cultivars were selected based on previous studies that indicated high levels of susceptibility to this pathogen; 'Strawberry Festival' is commonly used by University of Florida researchers as a susceptible check in their disease management trials (Mertely et al., 2015). Each cultivar+inoculation method was replicated on five beds 150 ft long, except for the 'Strawberry Festival', which was only grown on a single bed per inoculation method (three beds total) due to limited availability. On the non-infested side of the field, 'Albion' and 'San Andreas' were replicated on five beds while 'Strawberry Festival' was planted on a single bed (Figure 1).

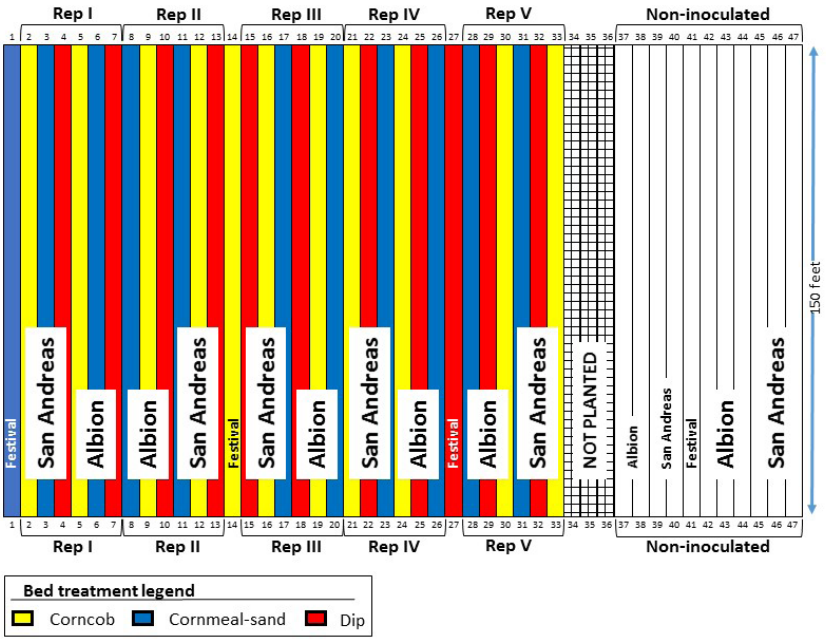

Figure 1. Plot map of 1-acre, *Macrophomina*-infested field site at Cal Poly San Luis Obispo.

Macrophomina phaseolina Inoculum Preparation Methods

Dip Treatment

This inoculum was produced by growing three local isolates of *M. phaseolina* on 12 Petri plates of potato dextrose agar (PDA) for one month. Colonized agar plates were homogenized in a blender with 2.5 liters of 0.35% water agar to produce a slurry (T. Gordon, personal communication); 125 ml of this slurry was used to inoculate a total of six bare root transplants for 5 minutes and then planted in the field.

Cornmeal-sand Treatment

A homogenized 1.1:0.4:0.4 sand, cornmeal and deionized water mixture was autoclaved for one hour on two separate days and then inoculated with mycelial plugs of three different isolates of M. phaseolina. Inoculated cornmeal-sand was incubated at 77 F (25 C) and shaken daily to aid the uniform colonization of the mixture (Mihail, 1992).

Corncob Granule Treatment

This inoculum was produced using moist corncob granules autoclaved twice and then inoculated. After inoculation, the granules were shaken daily to aid colonization.

The cornmeal-sand and corncob granule treatments were applied by placing 5 grams of inoculum in the transplant holes the day after transplanting. Due to limits on the amount of inoculum produced, only the outside plant rows of each bed were inoculated (approx. 244 plants/bed), leaving the inner two rows non-inoculated. This served as a continuous control along each bed and provided a means of measuring disease spread (i.e., from outer to inner plant rows) over time.

Treatments were assessed by counting plant mortality nine times, beginning August 18 (six weeks after transplanting) and ending on November 23.

On May 13 and 16, 2016, healthy strawberry plants from the inside plant rows of the beds and plants that did not die from the previous inoculations were stab-inoculated using *M. phaseolina* inoculated toothpicks. Plant mortality was assessed nine weeks later.

Results

All cultivars were susceptible to crown rot and each of the inoculation techniques was effective, but differed in the rate of disease progress. Plant mortality data was evaluated with a two-factor ANOVA with replication and a post hoc Tukey HSD test (Table 1). All inoculation treatments were found to be significantly different ($p=0.05$), with the dip inoculation method causing the highest mortality across cultivars (98%), followed by the cornmeal-sand (68%) and corncob (42%) treatments. Irrespective of inoculation method, 'Albion' (54% mortality) tended to be more susceptible to *M. phaseolina* than 'San Andreas' (50% mortality) and 'Strawberry Festival' was the most susceptible (73% mortality). There was no interaction effect from the variety on the inoculation treatments. Although 'Strawberry Festival' was not included in the statistical analysis due to lack of replication, it appeared to be the most susceptible cultivar in the trial expressing disease symptoms as early as three weeks after inoculation and exceeded 'Albion' and 'San Andreas' in percent mortality throughout the entire experiment and regardless of inoculation method (Figure 2).

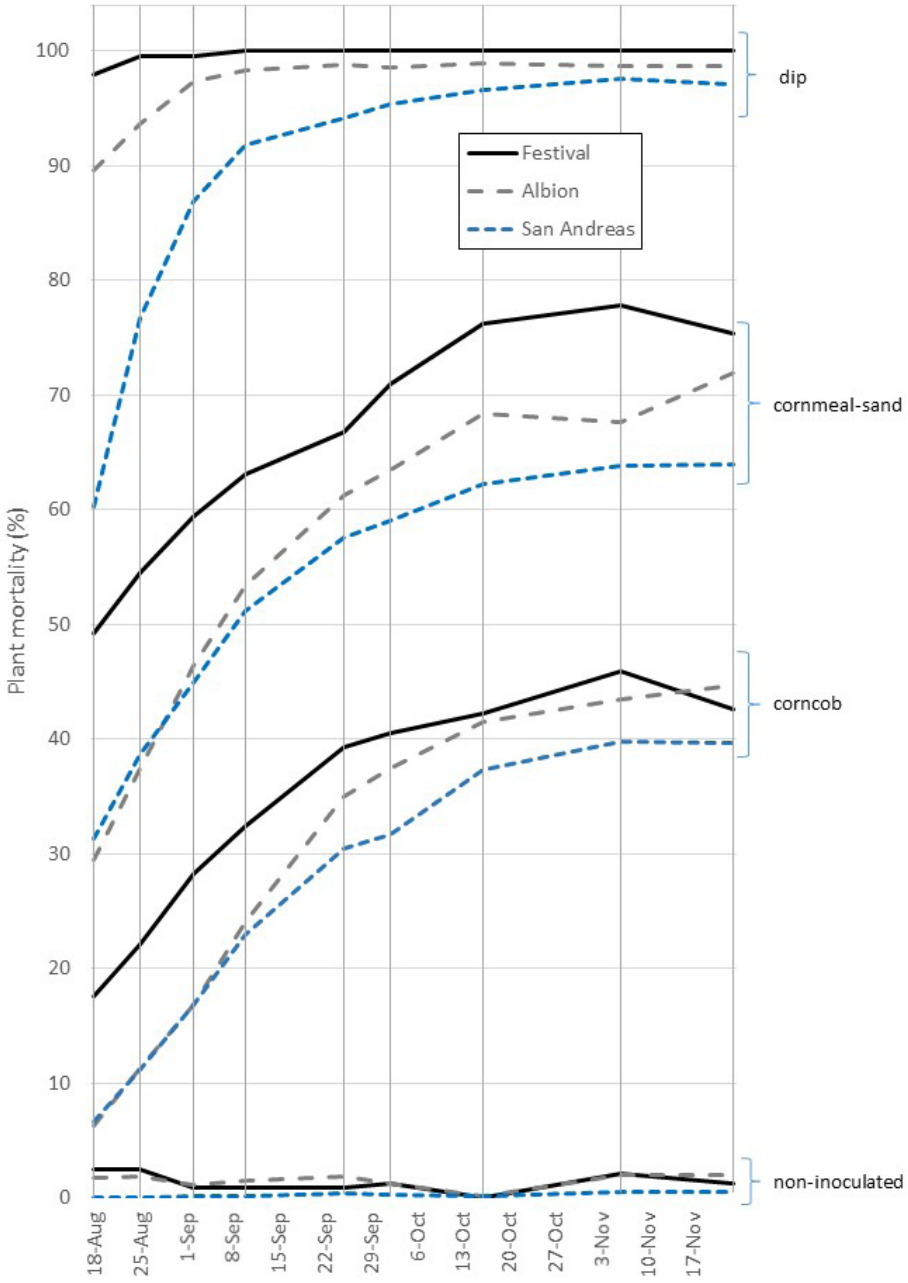

Figure 1. Percent mortality for each cultivar and inoculation method.

Table 1. Mean percent plant mortality for three inoculation methods at 140 days post inoculation.

	Mean plant mortality (%)	
Treatment	**'Albion'**	**'San Andreas'**
Dip	99 a *	97 a
Cornmeal-sand	72 b	64 b
Corncob granules	45 c	40 c
Non-inoculated	1 d	1 d

* Means followed by the same letter were not significantly different from each other within or between columns using post hoc Tukey HSD (p=0.05)

For the late-season toothpick stab inoculation, plant mortality counts were 30% for inoculated plants compared to 7% for non-inoculated plants; there was almost no spread (<1%) of disease from outer plant rows to inner rows.

Discussion

Macrophomina crown rot was successfully established in plants grown in field 35b on the campus of Cal Poly. All inoculation techniques were successful, with the dip treatment having the most rapid symptom development, followed by the cornmeal-sand and corncob methods. The cornmeal-sand inoculation method appears to be the most suitable for future field work based on the intermediate rate of disease progress. Cultivars 'Strawberry Festival', 'Albion' and 'San Andreas' (listed in descending order of susceptibility) responded as expected, and all were susceptible to the pathogen. Stab inoculation using *Macrophomina*-colonized toothpicks was less effective than anticipated, likely because of the large plant size and woody crown tissue at this stage of their growth. Only a few non-inoculated plants became diseased, even when they were only a few inches from inoculated plants. We will continue to inoculate plants in future years to achieve consistent disease pressure since it will take many years to build up inoculum in the soil. This field site will be used to evaluate host plant resistance and other management tactics for control of *Macrophomina* crown rot in the coming production seasons.

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