Management of Diseases caused by *Fusarium oxysporum* f. sp. *fragariae* and *Macrophomina phaseolina*

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Summary

Knowledge on the ecology and lifecycle of soilborne pathogens is prerequisite to designing fumigant free disease management strategies. This knowledge enables us to design techniques that disrupt critical life stages and reduce future disease severity. We propose two main objectives to investigate the ecology and lifecycles of *Macrophomina phaseolina* (cause of charcoal rot) and *Fusarium oxysporum* f. sp. *fragariae* (cause of Fusarium wilt). The first objective is to investigate the potential for *M. phaseolina* to cause disease, or grow asymptomatically, on common rotation and cover crops. This pathogen has a host range of over 500 plant species, and proliferation on other crops would lead to increased disease severity when strawberries are re-planted. In addition to symptom assessment, we will assay plants for asymptomatic infections which indicate the pathogen is able to build inoculum on host tissues.

The second main objective is to investigate the extent to which removing or fragmenting pathogen-infested crowns can reduce future disease incidence. Past work has shown population growth on infested crown tissues to be an important mechanism for persistence of *F. oxysporum* f. sp. *fragariae*. Fragmentation can be achieved by multiple tractor implements already present in grower fields. This could expose crown tissues to the soil environment so they decompose more quickly and are more effectively disinfested by soil treatments. Devastating soilborne pathogens such as such as *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *fragariae* are not just pathogens of strawberry, they have diverse ecological roles in soil and through interactions with non-susceptible plants. The proposed objectives will provide new information to leverage these interactions for reduced abundance and disease. Our research will address the California Strawberry Commission research priority 1 – Farming without fumigants, and research priority 2 – Management of soilborne diseases.

Introduction

A key, unanswered question for management of charcoal rot of strawberry, caused by *M. phaseolina*, is whether this strawberry pathogen causes disease on other crop species. Isolates of *M. phaseolina* typically have a broad host range and hundreds of crop species are reported as susceptible (Dhingra and Sinclair, 1977). This host range includes many cover and rotation crops that are commonly grown in strawberry production regions. This pathogen could proliferate if susceptible species are planted in infested fields (Gupta et al., 2012). Increased pathogen abundance would limit the efficacy of disease management strategies such as fumigation and tolerant cultivar selection.

Differences are likely to exist in rotation crop susceptibility to the genotype of *M. phaseolina* causing disease on strawberry. Past work has shown some degree of host specificity within this species (Su et al., 2001). For example, soil abundance of soybean-associated isolates declined during corn rotation, while abundance of corn-associated isolates increased (Pearson et al., 1987). Furthermore, the ability to cause disease on strawberry does not appear to be typical for *M. phaseolina*; only one genotype of M. phaseolina is consistently isolated from diseased strawberry plants in California (Burkhardt et al., 2018). This strawberry-associated *M. phaseolina* genotype was not able to cause disease on five species of cover crops in greenhouse trials (Koike et al., 2016). These data suggest specialization to the strawberry host has occurred, but the range of susceptible hosts has not been elucidated.

In addition to characterizing pathogenicity on additional hosts, trials should consider the influence of environmental conditions on disease symptom development. Hot and dry environments greatly favor disease development in many *M. phaseolina* pathosystems, including strawberry (Gupta et al., 2012). Conducting pathogenicity trials under field conditions will greatly improve our understanding of the potential for pathogenicity on these hosts. Spence Ranch near Salinas, CA would be an ideal field site for these experiments, because it is relatively inland and has greater temperature fluctuations and daily highs. Additionally, Spence Ranch regularly has moderate wind-speeds in the afternoon that increase transpiration and water stress. Experimental conditions can never completely match those observed in production fields, so we will also plan to identify 1 infested fields and assay rotation and cover crops for infection by this pathogen in subsequent seasons. The synthesis of experimental and field survey data will establish which, if any, common crop species are hosts of *M. phaseolina*.

Understanding the impact that alternate plant hosts may have on soilborne pathogen populations is critical to designing fumigant-free disease management strategies. Strawberries are grown in high value agricultural areas where diverse crops are grown, and many strawberry growers use cover crops to improve soil tilth. Many cover crops have been identified as susceptible to *M. phaseolina* (Dhingra and Sinclair, 1977). Proliferation on these alternate hosts could mean the difference between sustainable strawberry production and disease outbreaks.

Knowledge of pathogen ecology can reveal lifecycle stages that are critical for survival or reproduction. Reproduction of *F. oxysporum* f. sp. *Fragariae*, a key stage of its lifecycle, was recently identified in the strawberry crown tissue (Henry et al., 2019). Populations of this pathogen become highly concentrated in strawberry plant tissues, with population densities more than 1,000 times greater than what is typically observed in highly infested field soil (Henry et al., 2019). If infested tissues are returned to soil, they will significantly increase the levels and longevity of *F.o. fragariae* in soil. The goal of our research is to investigate management techniques that will disrupt this important life cycle stage, so that pathogen survival and disease severity are reduced.

Fumigation can be used to assess the extent of protection of the pathogen in these tissues. In an initial investigation during the 2018 season, we found 65% of infested, whole crowns buried 10 inches below the injection depth (20-inch total depth) remained infested with *F.o. fragariae* after fumigation (10-11 inches, broadcast applied, maximum application rate chloropicrin). Whole crowns buried at ~10 inches received a high dose of fumigant, yet 22% of these crowns remained infested after fumigation. In contrast, when *F.o. fragariae* propagules are soilborne (i.e., not protected inside tissues), near-complete eradication has been consistently observed. These data show that protection by host tissues increases longevity of pathogen propagules in soil through adverse conditions such as fumigation.

Strawberry crown tissues degrade slowly and can be recovered from fields years after a strawberry crop has been grown. Infested crowns assayed one year after burial showed no evidence of a reduction in pathogen population (Henry et al., 2019). These tough tissues are likely to protect the pathogen from adverse soil environments, so it is especially important to manage inoculum produced on these tissues. Common sense tells us that removing infested crowns will reduce inoculum, and that fragmenting crowns will accelerate decomposition and make soil disinfestation treatments more effective. Our proposed research will provide a cost-benefit analysis to enable informed decisions regarding deployment of these tactics. Initial analysis of the cost of these treatments suggest they could be feasible if applied on an as-needed basis. Plant removal and tissue fragmentation are relatively inexpensive (estimated $200 to 1,000 per acre), but it is unknown whether they will lead to improvements in yield that justify the expense.

In addition, there has been little research on the effects on pathogen eradication when 1,3-dichloropropene (i.e., Telone) is added to chloropicrin fumigation. 1,3-dichloropropene is often included in fumigation mixtures such as PicClor 60 (60% chloropicrin, 40% 1,3-dichloropropene) or TriForm 80 (80% chloropicrin, 20% 1,3-dichloropropene). It would be beneficial to all growers who fumigate to know whether there is any improved disease suppression when 1,3-dichloropropene is added to the mixture. If chloropicrin alone works just as well, it could save growers money to avoid applying another fumigant.

An increasing number of fields are being reported with moderate to severe incidence of Fusarium wilt of strawberry after pre-plant flat fumigation with maximum rate chloropicrin. It is likely that survival on host tissues contributes to these outbreaks, but this has not been specifically investigated. In organic or non-fumigated production systems, persistence on tissues could increase the interval of rotation needed for fields to be suitable for strawberry production. There is a critical need for research on managing pathogen-infested plant tissues

Methods and Materials

Experiments to assess colonization of rotation and cover crops by M. phaseolina

We investigated the ability for strawberry-pathogenic strains of *M. phaseolina* to asymptomatically infect rotation and cover crops. These experiments were conducted in a field that had a 'naturally' occurring infestation of *M. phaseolina*, where >50% plant mortality was observed in the 2018-2019 strawberry season. We define rotation crops as crops grown for harvest and sale, whereas cover crops are grown and incorporated into the soil without harvest.

During spring of 2020, we transplanted eight rotation crops (broccoli, broccoli raab, cauliflower, kale, iceberg lettuce, romaine lettuce, spinach and cilantro) in 10-plant plots with six plot replicates arranged in randomized complete block design. Soil from each plot was collected at the time of planting and *M. phaseolina* was quantified by DNA extraction and qPCR (Burkhardt et al., 2018). Each crop was grown to maturity and observed for charcoal rot symptoms. At the time of harvest, we collected fine roots (i.e., less than 1 mm in diameter) from five plants per plot. These roots were washed and sonicated to remove soil. We quantified M. phaseolina from the soil collected off the surface of these roots (rhizosphere soil) with DNA extraction and qPCR. Quantifying *M. phaseolina* microsclerotia from the rhizosphere soil indicates how well it can colonize the root surface, which may be an important niche for survival. The dried root tissues were ground into a fine powder (425 µm square mesh pore size) and the microsclerotia per gram of root tissue was quantified with a Petri plating assay (Cloud and Rupe, 1991).

We also collected the taproots from all 10 plants in each plot, surface sterilized them in a 1% sodium hypochlorite (dilute household bleach) solution and plated them on NP-10 semi-selective medium. These plates were observed for *M. phaseolina* colonies, and crops were compared to determine those which harbor a greater and lesser abundance of *Macrophomina*.

Initial concentrations of M. phaseolina microsclerotia per gram of bulk soil were analyzed by ANOVA using the 'lm' function in R version 4.0.4). For the microsclerotia per gram of rhizosphere soil data, we adjusted for heterogeneity of variance by fourth-root transforming. Then, treatment differences were evaluated by constructing a linear model for the data (R package: "lm") and using the R package "emmeans" to conduct pairwise comparisons (using the Tukey's method for P-value adjustment).

In January and February 2020, we planted 16 varieties of cover crop (including 10 species in three plant families) in the Macrophomina-infested field at Spence Rd. This list includes the 'Summit 515' wheat variety that has shown potential to suppress *Macrophomina* in greenhouse studies. Each variety was planted in 10-plant plots (blocks) arranged in randomized complete block design with six replicate blocks per variety. At the time of maturity, we collected taproots from each plant, surface sterilized it in 1% sodium hypochlorite solution, plated it on NP-10 (semi-selective for *M. phaseolina*), incubated plates at 30°C for seven to 14 days, and scored each plant for the presence or absence of *M. phaseolina*.

In addition to these studies to assess infection rate, we planted combinations of the aforementioned cover crops into 50-foot sections of 80 inch beds with seeding rates that are typical for each mixture. These treatments included a legume/oat mixture, mustard mixture (*Sinapa alba* and *Brassica juncea*), 'Summit 515' wheat monocrop, and fallow (nothing planted). Each field treatment was established in six block replicates arranged in randomized complete block design. From two locations in each replicate of each treatment, we quantified soil *Macrophomina* populations before planting, at the time of tillage, and eight weeks post tillage by DNA extraction and qPCR. The results were analyzed to assess the population changes in soil that are associated with each crop.

Grower field surveys to investigate changes in the population density of M. phaseolina in strawberry fields over time

Fields in strawberry production with *M. phaseolina* disease outbreaks were identified during the first year of this study (2019-2020 growing season). Soil abundance of this pathogen was quantified by soil dilution plating on a modified potato dextrose agar medium and counting the resulting *M. phaseolina* colonies that grow (Cloud and Rupe, 1991). Disease severity was assessed on an ordinal scale of 1-5, where 1 means a completely healthy plant, and 5 means the plant is dead. Two 20' x 20' areas were marked by GPS measurement per field, and all plants in these areas were rated for disease severity.

Crown disinfestation by fumigation trial design

We replicated each fumigation treatment (PicChlor60 and TriChlor) at two ~1/2-acre blocks per trial. Within each ~1/2-acre block we established three locations where crowns (whole, half, and quarter-sized) were buried at 2-, 10-, and 20-inch depths. At each depth at each burial spot, there were 10 crown pieces per size (whole, half, quarter). We conducted this trial twice: at an *F.o. fragariae* infested field near Watsonville, CA and an *M. phaseolina*-infested field near Santa Maria, CA.

Treatments:

- Broadcast fumigation (two treatments):
    - Maximum rate PicClor60 (350 lb/acre chloropicrin, 233 lb/acre 1,3-dichloropropene)
    - Maximum rate TriClor (350lb/acre chloropicrin
- Crown sizes (three):
    - Whole crown (the lowest ~3.5 cm of the crown)
    - Half-crown (same size as whole crown, cut in half lengthwise)
    - Quarter crown (half-crowns cut again lengthwise)
- Depths:
    - 3 inches (7.6 cm)
    - 10 inches (25.4 cm)
    - 20 inches (50.8 cm)

Assessing pathogen survival through fumigation on crown tissues

Before fumigation we collected crowns from plants that died of *Fusarium* or *Macrophomina* (depending on the experiment). These were processed into whole-, half- and quarter-crowns and placed into mesh bags. The day before fumigation we buried crown fragments (10 per size per depth) at six locations per treatment and recorded their GPS locations. After the re-entry interval, we used GPS devices to recover crowns from the field. In the lab, we surface sterilized fragments in 1% sodium hypochlorite and plated them on selective media to test for the presence of the pathogen. For an untreated control we assayed six batches of 10 whole, half-, and quarter-crowns pre-treatment. Data were analyzed to determine the rate of disinfestation based on a pre-vs-post proportion of infested crowns for each fragment size.

Quantifying soil gas concentrations

With the assistance of the TriCal soil gas monitoring team, we used GPS technology to record the locations of burial. On the day of fumigation, the TriCal soil gas monitoring team inserted soil gas monitoring devices in the same locations where the crowns were buried. This team monitored soil gas concentrations of chloropicrin and 1,3-dichloropropene at the depths of 3, 10, and 20 inches, for the duration that the tarp was on the field (seven to nine days). Data were converted into the concentration of gas / time ("Ct") measurements prior to analysis.

Disease severity assessment

During the subsequent strawberry season, we assessed disease severity with drone imagery conducted in the early season (February and April; to assess plant establishment) and late in the season (around early August); to assess disease severity. These data were used to assess differences in plant establishment or disease severity between fumigant treatments.

Results

Rotation crop colonization by M. phaseolina

We assessed the concentration of *M. phaseolina* inoculum from all plots at the start of the experiment. DNA-based estimates of microsclerotia per gram of soil showed no significant differences by treatment (α = 0.05; Figure 1), indicating that the results would not be biased by differences in the naturally occurring inoculum. At the time of harvest (based on each crop's marketable maturity), we collected fine roots, rhizosphere soil, and taproots to assess M. phaseolina colonization of each crop. We observed the microsclerotia per gram of rhizosphere soil was significantly greater in the cauliflower treatment compared to that of all the other crops except the broccoli and Salinas lettuce (Figure 2). Interestingly, the crops with the highest rhizosphere concentration of *M. phaseolina* did not have the highest concentrations of microsclerotia on root tissues (Figure 3). Differences in microsclerotia per gram of root tissue were not statistically significant (Figure 3). While we could recover *M. phaseolina* from the fine roots of all crops tested, we only recovered *M. phaseolina* from 6.7% of both cilantro and spinach taproots.

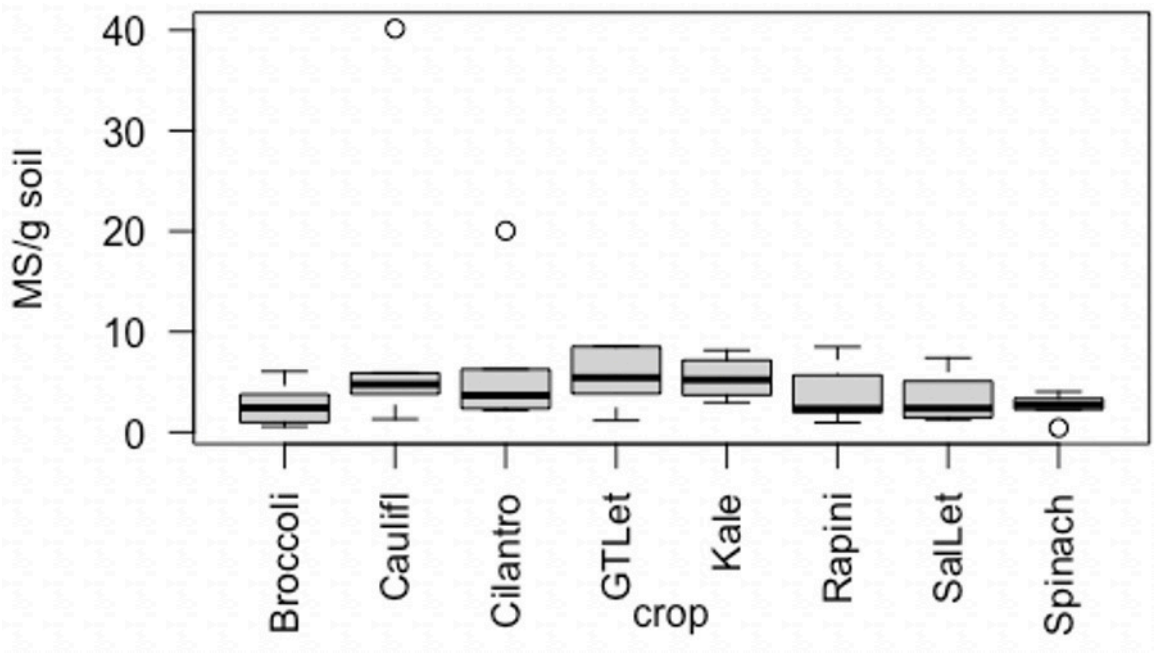

Figure 1. Boxplot depicting initial inoculum densities of *M. phaseolina* in rotation crop experiment plots (n = six per treatment). The y-axis depicts the number of microsclerotia per gram of soil ("MS/g soil"). The x-axis shows the eight crop treatments: broccoli, cauliflower ("Caulifl"), cilantro, romaine lettuce ("GTLet"), kale, rapini, iceberg lettuce ("SalLet"), and spinach.

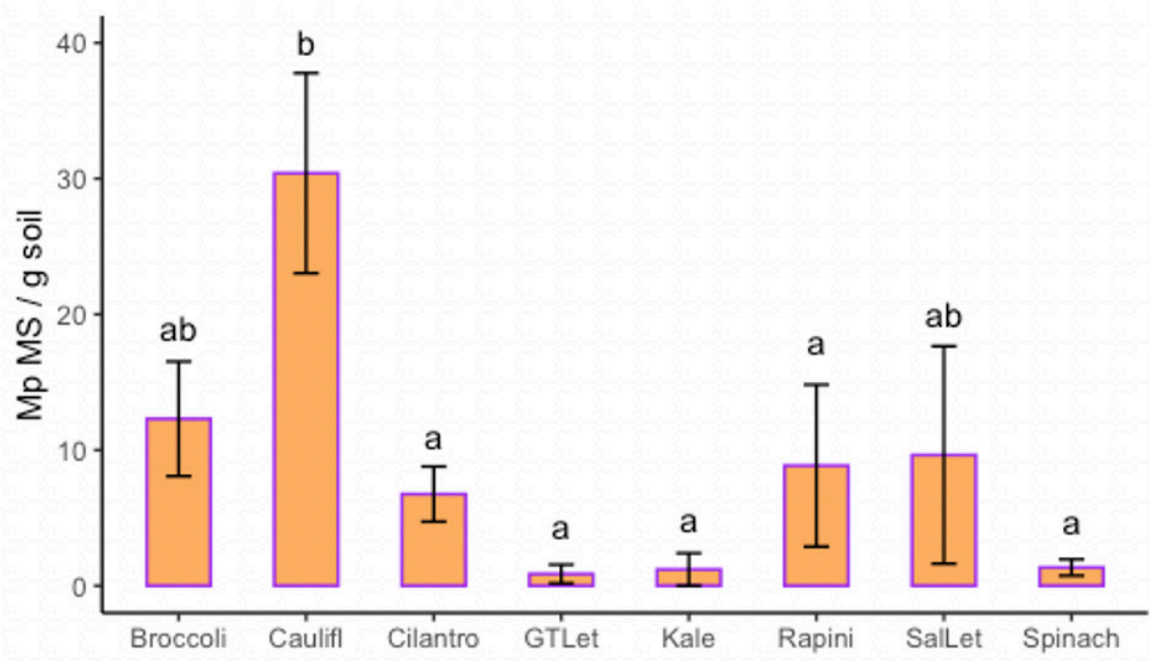

Figure 2. *Macrophomina phaseolina* microsclerotia per gram of rhizosphere soil from eight rotation crops. The y-axis depicts the untransformed values of microsclerotia per gram of soil ("Mp MS/g soil"), with letters indicating significant ($P<0.05$) pairwise differences determined by statistical analysis of fourth-root transformed data. The x-axis shows the eight crop treatments: broccoli, cauliflower ("Caulifl"), cilantro, romaine lettuce ("GTLet"), kale, rapini, iceberg lettuce ("SalLet"), and spinach.

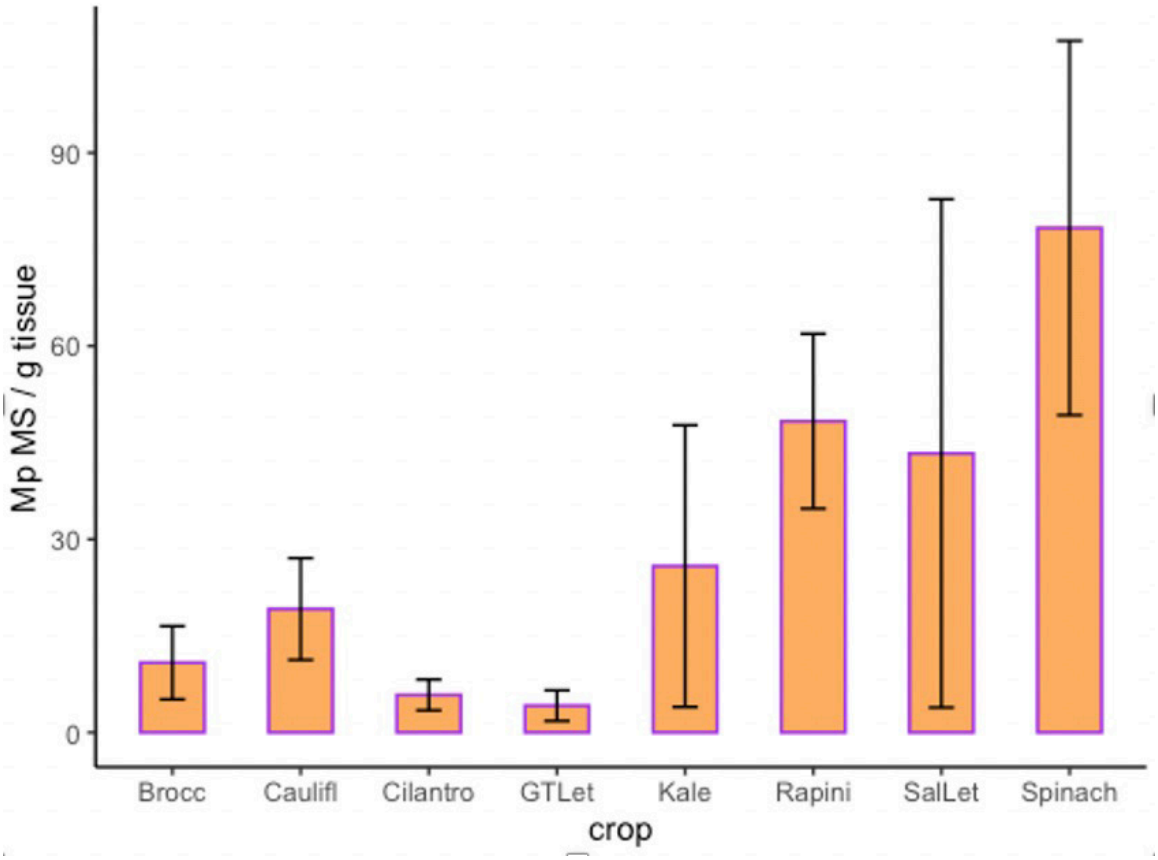

Figure 3. Concentration of *M. phaseolina* microsclerotia per gram of fine root tissues from eight rotation crops. Quantitative tissue plating was conducted on five plants per plot (six plots per crop). The y-axis depicts the concentration of *M. phaseolina* microsclerotia per gram of fine root tissue. Treatments are shown on the x-axis: broccoli ("Brocc"), cauliflower ("Caulifl"), cilantro, romaine lettuce ("GTLet"), kale, rapini, iceberg lettuce ("SalLet"), and spinach.

Cover crop colonization by M. phaseolina and changes in soil population densities after cover crop incorporation

We conducted two experiments in early 2020 to investigate the ability for *M. phaseolina* to colonize common cover crops and how this may impact soil populations of the pathogen (Figure 4). By assaying the taproots/crowns from 100 to 240 plants per cover crop variety, we determined that most cover crop taproots/crowns can be infected at a low rate by *M. phaseolina* (Figure 5). Overall, the taproots of plants in the mustard family (*Brassicaceae*) had the highest rate of infection at 5%, whereas plants in the bean family (*Fabaceae*) were on average infected at a rate of 2.5% (Figure 5). Remarkably, we recovered *M. phaseolina* from only one plant out of over 1,000 cereal plants that were assayed in these experiments. This suggests that cereal crops are poor hosts for *M. phaseolina* and could be a good option for cover cropping *M. phaseolina*-infested fields.

Statistically significant differences in the amount of microsclerotia per gram of soil were not observed at any of the timepoints (Figure 6). However, it is notable that the fallow plot where the burr clover weed grew had among the highest population densities of *M. phaseolina* in both experiments (Figure 6). The burr clover weed species also had high tissue population densities of *M. phaseolina* from partially decomposed plant debris recovered at 8 weeks post-tillage (Figure 7).

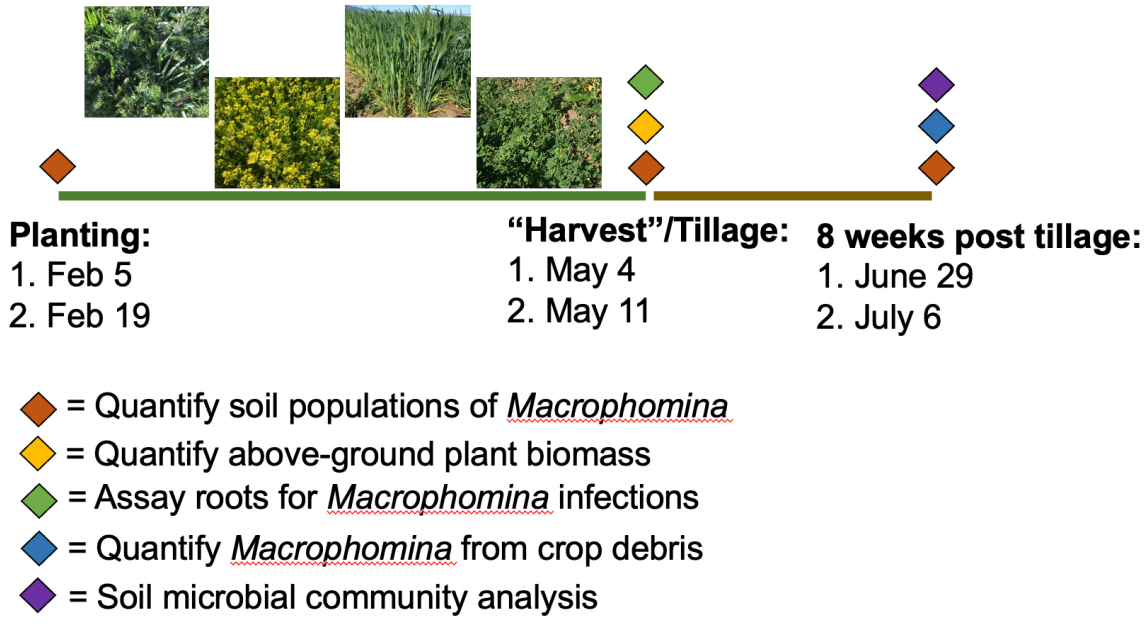

Figure 4. Timeline and data collection plan for cover crop experiments. The two experiments were planted on February 5 and 19, 2020, harvested (roots collected, tilled into soil) on May 4 and 11, and a post-tillage soil sampling collected on June 29 and July 6. Colored diamonds depict the assays conducted on samples collected at each of these timepoints.

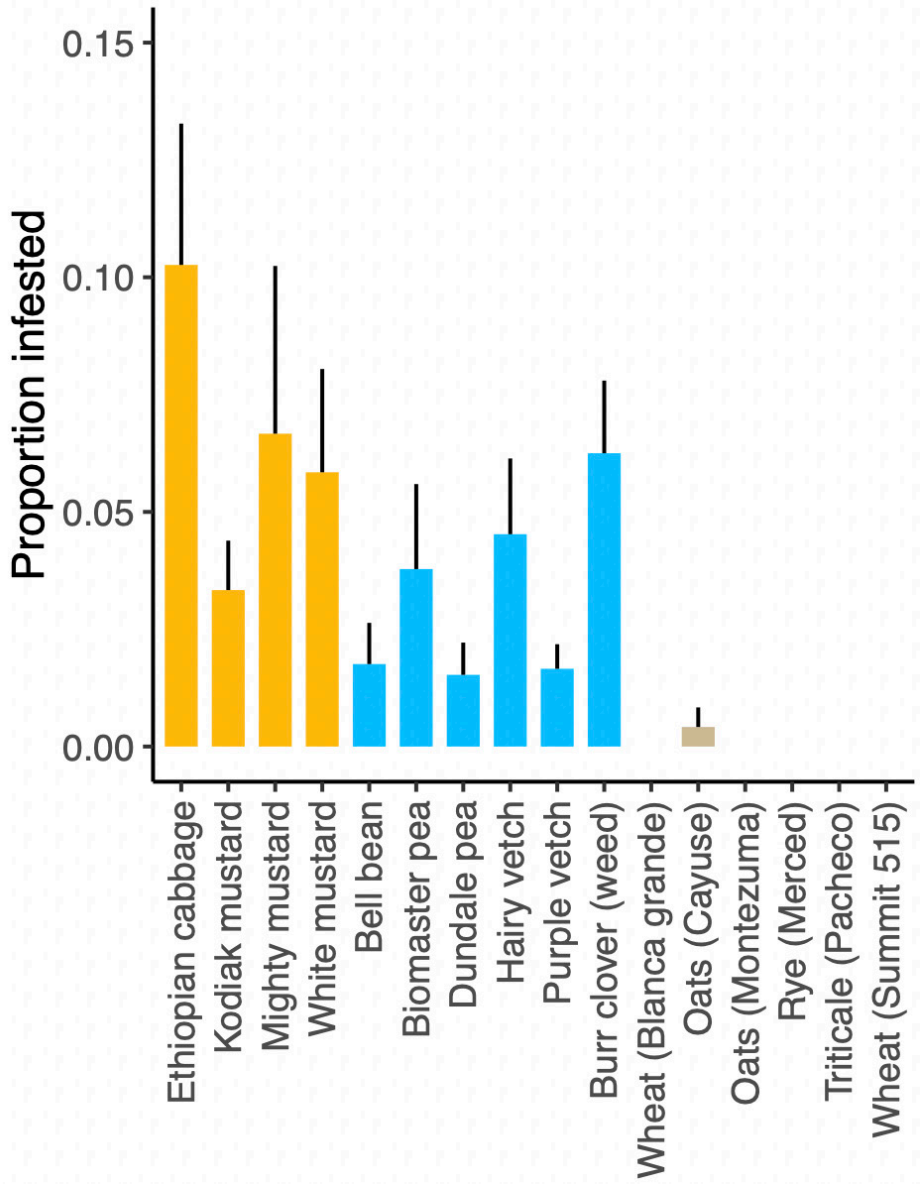

Figure 5. The proportion of each cover crop variety from which *M. phaseolina* was recovered. Each cover crop variety is shown individually on the x-axis and color-coded by plant family, where yellow = *Brassicaceae*, blue = *Fabaceae*, and brown = *Poaceae*. The y-axis depicts the average proportion of plants that were infected by M. phaseolina, error bars depict 1x standard error.

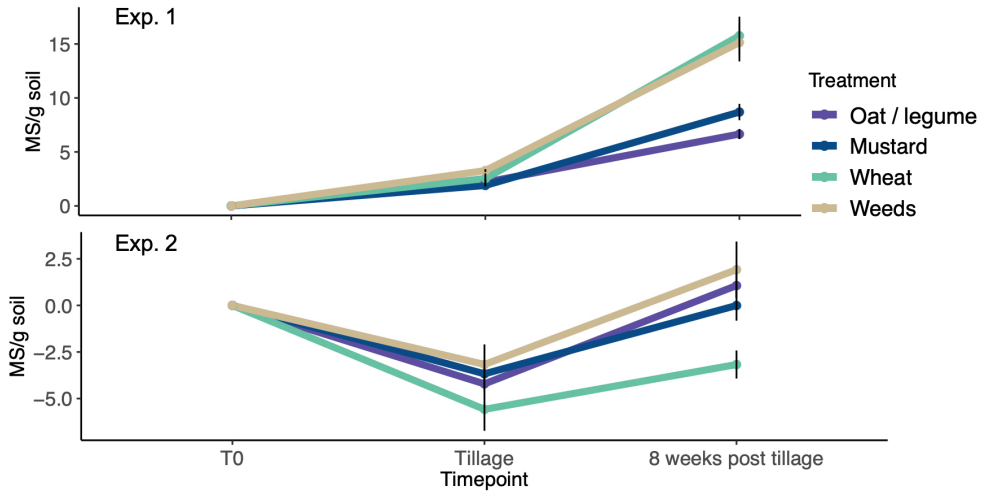

Figure 6. Changes in soil population density of *M. phaseolina* between the time of planting, tillage, and eight-weeks post-tillage. The y-axis depicts the average number of microsclerotia per gram of soil ("MS/g soil") estimated from quantitative PCR assays. The x-axis depicts the three timepoints: time of planting ("T0"), time of tillage ("Tillage"), and eight-weeks post-tillage. Treatments are color-coded and the legend provided on the right side of the figure.

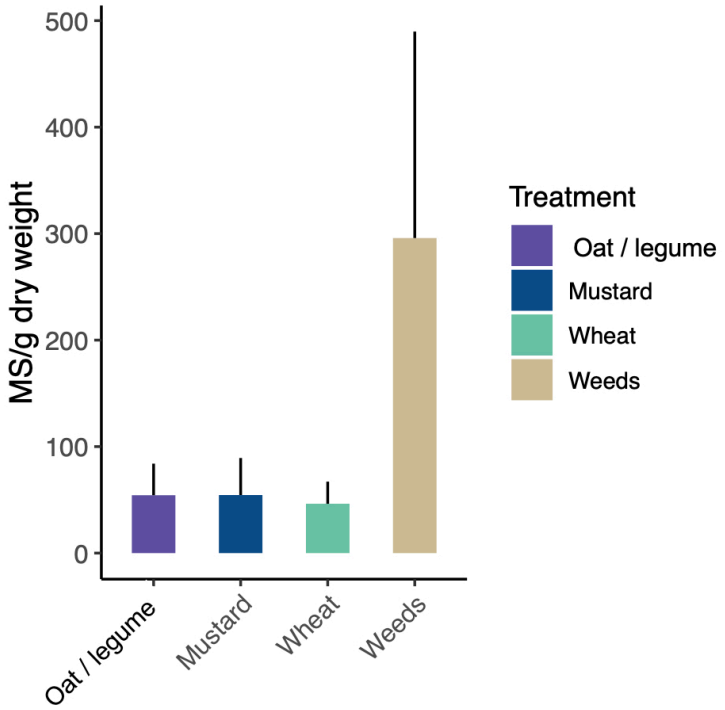

Figure 7. Average concentration of microsclerotia per gram in partially decomposed plant tissue recovered at eight weeks post tillage. Plant debris that had started to decompose was collected from soil samples taken at eight weeks post cover crop incorporation. These tissues were ground and quantitatively assayed for *M. phaseolina* microsclerotia. The y-axis depicts the average concentration of microsclerotia per gram of dry weight ("MS/g dry weight"), and the treatments are shown on the x-axis.

Changes in soil population densities of M. phaseolina over time

Four fields were selected for this study and the initial soil inoculum density assessed. The average microsclerotia per gram of soil ranged from 0.5 to 8 at the time of sampling and the initial mortality of the closest 48 plants ranged from 5% to 73%. Only one timepoint was collected in year 1 of this study.

Crown disinfestation of F. oxysporum f. sp. fragariae and M. phaseolina by fumigation and associated soil gas concentrations

We conducted crown disinfestation trials at a *F. oxysporum* f. sp. *fragariae*-infested field in Watsonville and a *M. phaseolina*-infested field in Santa Maria. Crowns were either left whole or cut into half- or quarter-crown pieces (Figure 8) and buried at 2, 10, and 20 inch depths. Chloropicrin and 1,3-dichloropropene gas concentrations were lowest at the 20-inch depth at both sites (Figure 9). Chloropicrin concentrations at the 20-inch depth were not significantly different between treatments, indicating that 1,3-dichloropropene is not affecting movement of chloropicrin in the soil (Figure 9).

Disinfestation efficacy was very high at the *F. oxysporum* f. sp. *fragariae*-infested field in Watsonville (Table 1). Overall, there were not significant differences between treatments (PicChlor 60 vs. TriChlor) in disinfestation efficacy. Consistent with the trend observed for gas concentration, the greatest survivorship occurred at the 20-inch depth for both treatments. There was numerically less survivorship on quarter-sized crowns than half or whole-crowns (in either fumigant treatment). Replication from years 2 and 3 of this project will enable a robust statistical testing of this observation. Although the fumigation appeared to be highly effective at disinfesting crowns at this field, we observed severe disease in the subsequent season. A drone flight on July 27 did not show a difference in plant health between PicChlor60 and TriChlor plots based on canopy cover measured by NDVI (0.744 and 0.775 for PicChlor60 and 0.727 and 0.747 for TriChlor).

The soil at the *M. phaseolina*-infested field in Santa Maria was below the label specification for soil moisture during flat fumigation. This could affect the efficacy of the results. Despite this, we observed a low survivorship of *M. phaseolina* on crowns after the flat fumigation treatment with PicChlor60 (Figure 10). This was significantly lower than the flat fumigation treatment with TriChlor, but not significantly different than bed fumigation with PicChlor60 (Figure 10). Both bed and flat PicChlor60 treatments were significantly more effective at eradicating M. phaseolina from crowns than any application method of TriChlor (Figure 10). Consistent with this observation, the canopy cover (measured by mean NDVI) in blocks treated with PicChlor60 was greater at the end of the season than blocks treated with TriChlor; mean NDVI was 0.821, 0.769, and 0.814 for PicChlor60, whereas mean NDVI was 0.753, 0.675, and 0.738 for TriChlor (Figure 11). This suggests that the addition of 1,3-dichloropropene, which is typically regarded as a nematicide, also improves disinfestation of the soilborne pathogen, *M. phaseolina*. Additionally, an extra application of KPAM after TriChlor bed fumigation did not significantly improve crown disinfestation efficacy or NDVI values at the end of the season (Figure 11).

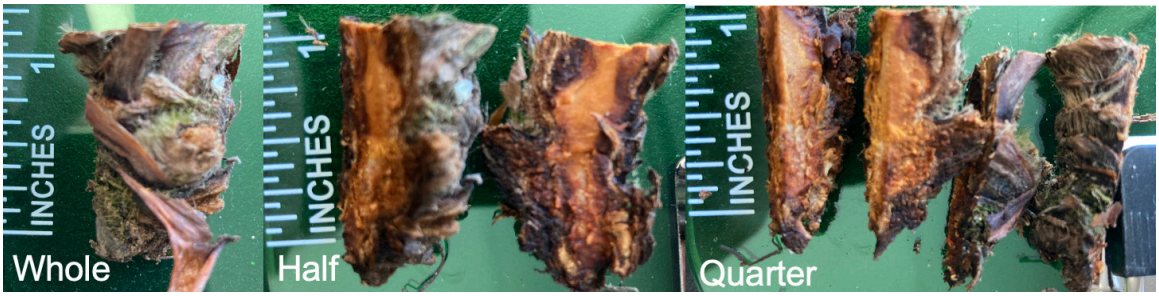

Figure 8. Images of the three sizes of crowns used in disinfestation trials. The basal ~1 inch of crown was trimmed of adhering roots for the whole crown size. This was cut in half vertically for the half size, and vertically again for the quarter crown size.

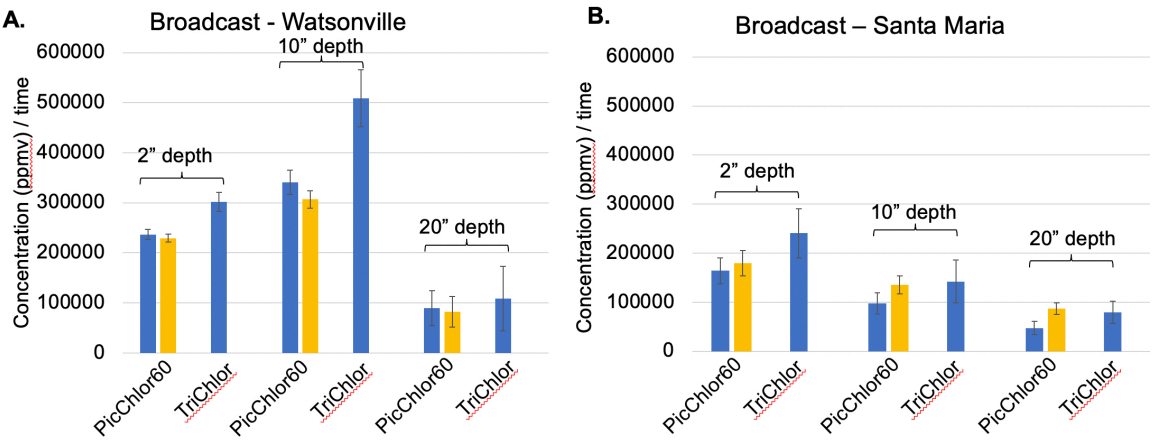

Figure 9. Gas concentrations at the depths where crowns were buried in flat fumigation trials. The trials were conducted in a *F. oxysporum* f. sp. *fragariae* infested field in Watsonville (**A**) and a *M. phaseolina* infested field in Santa Maria (**B**). The y-axes depict the average concentration (measured in ppmv) over time for each fumigant. The x-axes show the active ingredient chloropicrin (blue; "Pic") and 1,3-dichloropropene (yellow) for the two fumigant treatments (either PicChlor60 or TriChlor). Measurements were taken at 2-, 10-, or 20-inch depth.

Table 1. Disinfestation efficacy for *Fusarium oxysporu*m f. sp. *fragariae* (Fof) from crown tissues by fumigation.

Treatment[a]	Depth[b]	Fragment size[c]	# Infested[d]
PicChlor60	2 inches	Whole	0
PicChlor60	2 inches	Half	0
PicChlor60	2 inches	Quarter	0
TriChlor	2 inches	Whole	1
TriChlor	2 inches	Half	0
TriChlor	2 inches	Quarter	0
PicChlor60	10 inches	Whole	0
PicChlor60	10 inches	Half	1
PicChlor60	10 inches	Quarter	0
TriChlor	10 inches	Whole	1
TriChlor	10 inches	Half	2
TriChlor	10 inches	Quarter	0
PicChlor60	20 inches	Whole	3
PicChlor60	20 inches	Half	1
PicChlor60	20 inches	Quarter	0
TriChlor	20 inches	Whole	2
TriChlor	20 inches	Half	2
TriChlor	20 inches	Quarter	1

a Crowns were either broadcast fumigated with maximum rate TriChlor (350 lb chloropicrin per acre) or PicChlor60 (350 lb chloropicrin per acre plus 233 lb 1,3-dichloropropene per acre).

b The depth at which crowns were buried (either 2-, 10-, and 20-inches from the surface of the soil).

c The size of crown assayed. Crowns were either left whole or cut vertically in half or quarter-crown pieces.

d The number of crowns still infested after fumigation out of 60 total assayed per combination of fumigant, depth, and size treatments.

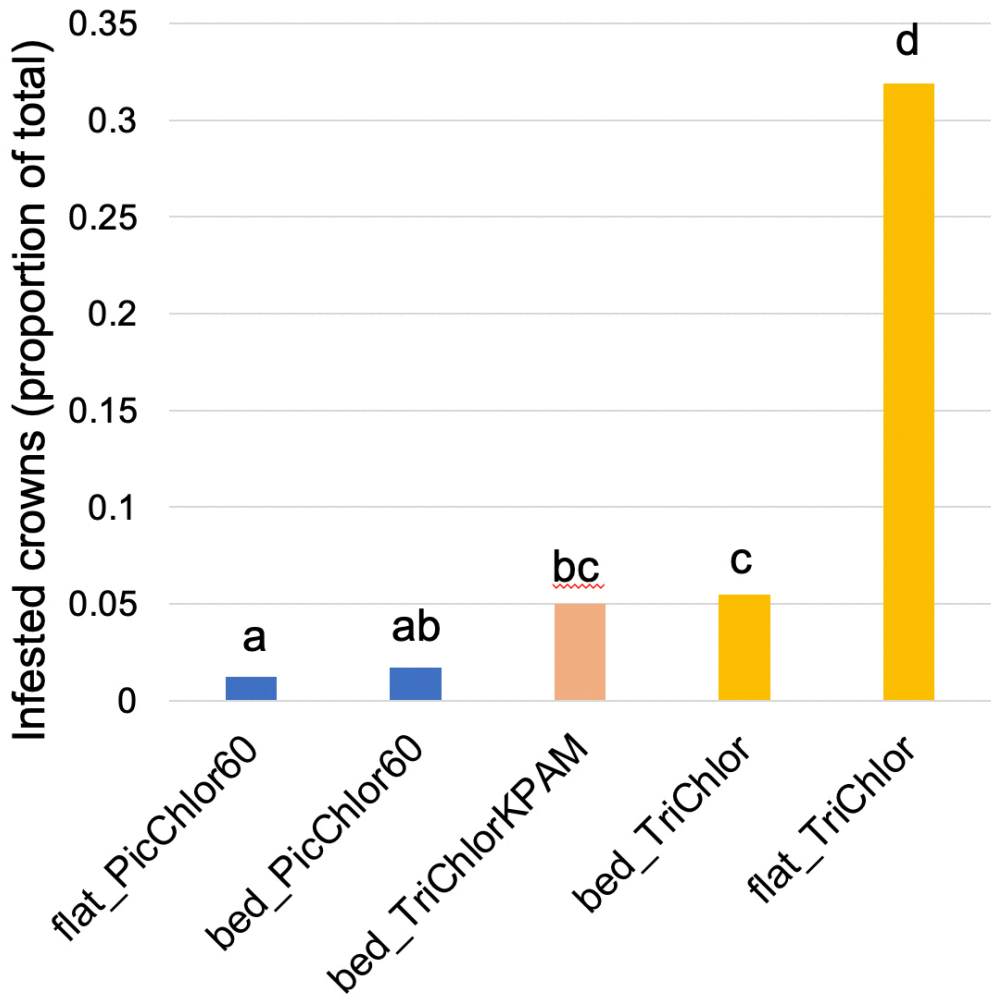

Figure 10. Survival of *M. phaseolina* through bed and flat fumigation. These results depict the proportion of crowns that remained infested with *M. phaseolina* after soil fumigation across all crown sizes and depths. Different letters above the bar graphs signify significant differences by a Chi-squared test adjusted for multiple comparisons by the Marascuilo procedure.

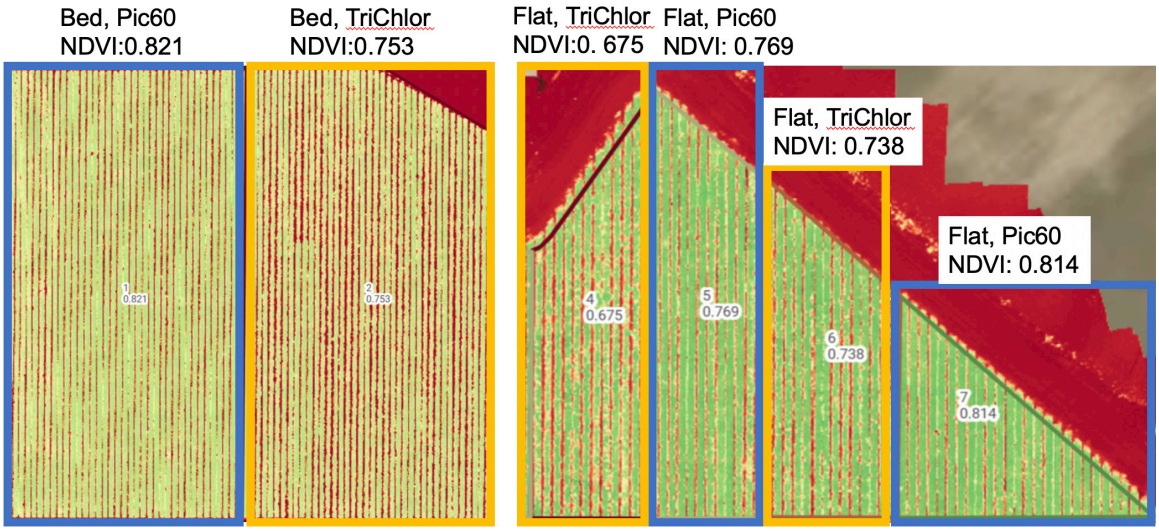

Figure 11. Drone imagery and NDVI values from bed and flat fumigation trials in a *M. phaseolina*-infested field in Santa Maria. The left panel shows imagery from the bed fumigation trial, where each block was approximately 1.5 acres (one block per treatment), and the right panel shows imagery from the flat fumigation trial, where each block was approximately 0.5 acres (two blocks per treatment). Areas treated with PicChlor60 are outlined in blue, whereas TriChlor treated areas are outlined in yellow. The mean NDVI across each block is listed below the treatment label.

Discussion

These results from the first year of study support previous reports of host specificity for the *M. phaseolina* genotype affecting strawberry in California. Furthermore, we observed that *M. phaseolina* could asymptomatically infect the fine roots of the rotation crops we tested and become enriched in the rhizosphere soil. Because we did not recover *M. phaseolina* from the taproots of most crops, it is likely that infections were localized on fine roots. Systemic colonization (evidenced by taproot infections) was only achieved at a low rate in spinach and cilantro. Because these crops can be systemically colonized by M. phaseolina they could be a poor choice for rotation crops in a *M. phaseolina*-infested field. In Cauliflower also appears to be a good host for M. phaseolina, because the levels on fine roots and rhizosphere were relatively high and may also want to be avoided in *M. phaseolina*-infested fields. Replication of this experiment in years 2 and 3 of this project is necessary to determine if the trends observed are consistent across multiple trials.

Similar to our observations with rotation crops, the cover crops we tested did not become symptomatic with charcoal rot disease. This result was surprising, given that many of these cover crops have elsewhere been reported as susceptible to charcoal rot (Dhingra and Sinclair, 1977), and further supports the hypothesis that the strain of *M. phaseolina* that is commonly affecting strawberry is host-specific. Asymptomatic infections of cereal species were extremely rare, and therefore cereal varieties would be good choices for cover cropping in *M. phaseolina*-infested fields. Although the 'Summit 515' variety of wheat has previously been reported to suppress charcoal rot, we did not find any effect of this treatment on the soil population densities of *M. phaseolina*. If this wheat variety indeed has an effect on charcoal rot disease, it is likely to be mediated by the microbial community, rather than by a direct effect on *M. phaseolina* population densities in soil. Further work is needed to test this hypothesis.

Because the first year's sampling occurred during the end of the strawberry season, we can compare the severity of disease at nearby plants to the esults suggest that the inoculum density of *M. phaseolina* required to cause disease is very low, and less than 1 microsclerotia per gram of soil. In one field, only 1.6 microsclerotia per gram of soil were recovered in an area with 73% mortality. Further tracking of these fields and others is essential to determine how generalizable these results are across fields.

The results from our first year of study suggest that fragmenting crowns to the quarter-crown size can improve disinfestation efficacy. However, additional replications of this trial are needed to have adequate data for testing the statistical significance of this association.

Unfortunately, neither fumigant (PicChlor60 and TriChlor, both broadcast at the maximum rate) were able to reduce Fusarium wilt disease below an economically damaging threshold. The broadcast PicChlor60 treatment was not significantly better than broadcast TriChlor for disinfesting crowns of *F. oxysporum* f. sp. *fragariae* or reducing disease caused by Fusarium wilt. Our observation that the fumigation was mostly effective at eradicating the fungus from crown tissues, but severe disease still developed suggests that low levels of surviving *F. oxysporum* f. sp. *fragariae* may be re-colonizing the soil after fumigation. This hypothesis can be tested in future experiments, and if true could be mitigated by post-fumigation treatments meant to stimulate microbial activity that could compete with this fungus in soil.

Both broadcast and bed-applied PicChlor60 were significantly better than broadcast or bed-applied Trichlor at disinfesting *M. phaseolina* from crowns and reducing plant losses from charcoal rot disease. This was an unexpected result, because 1,3-dichloropropene (the extra active ingredient in PicChlor60) is typically considered a nematicide with relatively weak fungicidal activity. It remains unknown if the same effect could be achieved with less 1,3-dichloropropene by applying a product such as Triform80, and this could be a useful line of inquiry for future investigations. For the time being, growers with *M. phaseolina*-infested fields would benefit from applying PicChlor60 instead of TriChlor for control of charcoal rot.

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