Effect of Soil Inoculum Density and Drought Stress on Fusarium Wilt and Charcoal Rot Disease Progress on Strawberry

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Summary

Fusarium wilt and Charcoal rot, caused by *Fusarium oxysporum* f. sp. *fragariae* and *Macrophomina phaseolina*, respectively, are important diseases of strawberry (*Fragaria × ananassa*) that affect the production of this crop in many locations throughout California. Both pathogens reside in soil, infect roots, grow into the plant's crown, and establish a systemic infection. Both diseases cause similar symptoms, including stunting, wilting, and dieback. Unfortunately, there are no effective management options to control soilborne diseases of strawberries other than soil fumigation. Therefore, identifying soil management alternatives for sustainable and effective disease control is important. The objectives of this study are to continue previous studies to determine the population densities of *F. o. fragariae* and *M. phaseolina* above which Fusarium wilt and Charcoal rot can be expected to cause damage to traditional and new UCD cultivars and evaluate the effect of drought stress on the development of the development strawberry soilborne diseases caused by *M. phaseolina* and *F. oxysporum* f. sp. *fragariae*. The soil around the root crown was inoculated with different inoculum levels. Plants were rated throughout the season for their mortality. Results showed that treatments that are not fully effective in eliminating the pathogen but when the inoculum level of *F. o. fragariae* is under 100 CFU/g in the soil and can still provide significant disease control. However, for Charcoal rot, results showed that in most cases, more than half of "moderately susceptible" strawberry plants would die when inoculum levels are higher than 447 CFU/g soil. On the other hand, the newly developed cultivar 'UCD Mojo' showed the best performance among the evaluated cultivars, with mortality lower than 20% and inoculum levels lower than 447 CFU/g. This study shows how different cultivars, 'Monterey', 'Frontera', 'UCD Victor', and 'UCD Mojo', respond to diverse inoculum levels in infested soil. Therefore, growers can make informed decisions on what cultivars to grow in infested soils. Additionally, the growers can also decide whether the percentage of losses at a low inoculum level is acceptable among the susceptible cultivars.

Introduction

Strawberry growers throughout California reported an increasing problem with collapsing strawberry plants since 2005. Symptoms consisted of wilting of foliage, plant stunting, and necrosis. Plants eventually collapsed and died, especially when they were subjected to environmental stresses or were bearing a heavy load of fruit. Either *F. oxysporum* f. sp. *fragariae* or *M. phaseolina* was recovered from symptomatic plants. Most of the affected fields no longer received the annual methyl bromide + chloropicrin pre-plant treatment, so disease development has been associated with this change in disease management practices. Currently, there is a need for non-fumigant control strategies for soilborne disease control due to increasing restrictions on fumigant use in California.

Differences in susceptibility to Fusarium wilt and Charcoal rot have been shown for several commonly grown strawberry cultivars (Gordon et al., 2016, Winslow et al., 2018). Previous studies have shown that cultivar 'Albion', highly susceptible to *F. o. fragariae* remains symptomless when inoculated at a low inoculum level (Pastrana et al., 2019). Knowledge of threshold levels for the development of Fusarium wilt and Charcoal rot would allow strawberry growers to make informed economic decisions for disease management to prevent and minimize production losses. Specific pathogen levels in the soil will inform in-field site and rate-specific fumigation, choice of resistant cultivars based on their tolerance to these pathogens, and cultural practices to suppress pathogen populations in soil. Treatments that are not fully effective in eliminating the pathogen can still provide disease control if inoculum levels are reduced below the threshold needed to cause disease depending on the chosen cultivar. Our preliminary results have shown that susceptible cultivars or with moderate susceptibility to *F. o. fragariae* and *M. phaseolina* remain symptomless when inoculated at a low inoculum level, and resistant or moderately resistant cultivars can show symptoms at a high inoculum level. In the last two years, UC Davis has released seven new cultivars, each with its own farming niche, with different levels of susceptibility and resistance to both diseases. For this reason, we consider it important to continue with this study, incorporating some of the new UCD cultivars.

Most of the soilborne diseases of strawberry often occurs in the summer during harvest season, coinciding with periods of high temperatures and drought stress. Several diseases whose severity is affected by drought have been described. Together, these biotic and abiotic stresses are seen as having a high impact because the combination of stresses strongly affects crop productivity (Pandey, 2017). However, little information is available on the relationship between the infection caused by *F. o. fragariae* and *M. phaseolina* and drought stress, including the effect of this stress on the plant response to the disease. A recent study (Sanchez et al., 2018) has shown that in plants under drought stress, the severity of the infection caused by *M. phaseolina* is higher than in fully irrigated plants, regardless of cultivar. However, cultivars that are able to maintain more stable water relations, despite irrigation deficit conditions, respond better to the disease.

The objectives of this project are to continue to determine the population densities of *F. o. fragariae* and *M. phaseolina* above which Fusarium wilt and Charcoal rot can be expected to cause damage to traditional and new UCD cultivars and evaluate the effect of drought stress on the development of soilborne diseases caused.

Materials and Methods

Effect of Soil Inoculum Density on the Development of Fusarium Wilt and Charcoal Rot on Different Strawberry Cultivars

Location. Experiments took place in artificially infested fields, one in and experimental field at Hansen Agricultural Research and Extension Center in Santa Paula, Ventura County, CA, and one at the UC Davis Armstrong Plant Pathology Research Fields in Davis, Solano County, CA.

Plant material. Bare-rooted strawberry transplants cultivars ‘Monterey’, ‘UCD Victor’, and ‘UCD Mojo’ were used in the Fusarium wilt trial in Davis. Cultivars ‘Fronteras’, ‘UCD Victor’, and ‘UCD Mojo’ were used in the Charcoal rot in Davis and Santa Paula. The cultivars used in these trials are classified as resistant to Fusarium wilt (‘UCD Victor’ and ‘Fronteras’), and susceptible to Fusarium wilt. (‘UCD Mojo’ and ‘Monterey’). Moderate resistance to Charcoal rot (‘UCD Mojo’), moderate susceptibility to Charcoal rot (‘UCD Victor’ and ‘Fronteras), and susceptible to Charcoal rot (‘Monterey’) (University of California, Davis, Strawberry Breeding Program).

Inoculum preparation. The inoculum for Fusarium wilt was prepared by growing a known pathogenic isolate of *F. oxysporum* f. sp. *fragariae* (GL1080, originating from Ventura County, CA) (Henry et al., 2017) on potato dextrose agar (PDA). Plates were incubated at room temperature (20 to 22°C) under continuous fluorescent light (24 h/day) for seven days. Fully colonized plates were blended in sterile water, and the resulting slurry was mixed with sterile sand. The sand mix was stirred periodically over several days until it dried out. The infested sand was mixed with sterile sand to adjust five different inoculum levels, Level 1 = 0 CFU/g, Level 2 = 100 CFU/g, Level 3 = 500 CFU/g, Level 4 = 1000 CFU/g, and Level 5 = 2000 CFU/g.

The inoculum for Charcoal rot was prepared by growing a known pathogenic isolate of *M. phaseolina* (GL1310, originating from Orange County, CA) on potato dextrose agar (PDA). Plates were incubated at room temperature (20 to 22°C) for seven days. Fully colonized plates were used to inoculate 250 ml plastic container with cornmeal and sand (145 mL of sand, 52.5 mL of cornmeal, and 52.5 mL of deionized water) and incubated for seven days at 30°C. Then, the sand mix was stirred periodically over several days until it dried out. The infested sand was mixed with sterile sand to adjust five different inoculum levels, Level 1 = 0 CFU/g, Level 2 = 25 CFU/g, Level 3 = 100 CFU/g, Level 4 = 1000 CFU/g, and Level 5 = 10000 CFU/g.

Establishment of the trials. Inoculated sands were buried in beds in both locations during fall 2021. One liter of soil was excavated from each planting hole at 6 to 8 inches deep. Nine hundred milligrams of this soil was mixed with 100 mg of inoculated sand and then returned to the planting hole. Strawberry plants were randomly planted a day after inoculation. The experiment was established and maintained according to standard grower practices throughout the seasons. Inoculum level treatments were performed in a randomized complete block design with four replicates of four plants per cultivar (three cultivars × five inoculum levels).

Disease assessment. At the end of the season, plants were rated for mortality in Ventura (June 2022) and in Davis (July 2022). Dead plants were brought back to the laboratory for further analyses to confirm the presence of the pathogen. Crown sections of each plant were rinsed with 0.1% Tween®20, followed by immersion in 70% ethanol for 20 seconds and 1% sodium hypochlorite for 60 seconds (Pastrana et al., 2019). Each sample was cultured on PDA media at room temperature under continuous fluorescent light for seven to ten days. Colonies of *F. oxysporum*

f. sp. *fragariae* and *M. phaseolina* were identified based on their morphologic characters.

Inoculum density in soil evaluation. Soil samples (0.8 inches in diameter, 8 inches long) were taken from the rhizosphere of each strawberry plant to quantify the inoculum density using soil-dilution plating in the laboratory.

For the Fusarium wilt trial, 10 g of soil per sample was stirred in a flask with 200 mL of 1% sodium hexametaphosphate solution for 5 min. Then, 10 mL of this solution was added to 90 mL of 0.1% water agar and stirred for 5 min. Aliquots of 400 µL were spread over the surface of Fusarium-selective medium plates (n=20) (Komada,1975). Plates were incubated under continuous fluorescent light for seven to ten days. Dilutions were repeated using two biological samples per plant. Colonies of *F. oxysporum* f. sp. *fragariae* were identified by morphology.

For the Charcoal rot trial, 10 g of soil per sample was blended with 250 mL of 0.5% NaClO solution at about 2000 rpm for 30 seconds and allowed to stand for 3 min. This blend and rest cycle (30 seconds, followed by 3 minutes) was repeated for a total of three times. Then, the content was passed through a sieve with a pore size of 44 µm (325 mesh) and washed with sterile distilled water to remove the NaClO. Then the washed soil was collected in 100 mL of sterile water. Aliquots of 400 µL were spread over the surface of *Macrophomina* selective medium plates (n=12) (NP-10, Kabir et al., 2004). Plates where incubated in the dark for 10 days at 30^{o}C. *Macrophomina* colonies were identified based on morphology.

Statistical analyses. An analysis of variance was performed, and the means were separated according to Fisher's least significant difference (LSD) (P = 0.05). The analyses were performed with the statistical software SigmaStat (Systat Software Inc., San Jose, CA).

Effect of drought stress on the development of Fusarium wilt and charcoal rot under greenhouse conditions. During June, 2022, 'Monterey' frigo plants were planted into a 1–L pot containing Sunshine® Mix #1. Plants were inoculated with 5 g of *M. phaseolina* infested sand, two months after planting, Additionally, a non-inoculated control was included as a treatment. Plants were well irrigated by watering daily to saturation under greenhouse conditions before applying the treatments. Eight weeks after planting, three irrigation levels were applied, and plants were irrigated daily with rates of 25, 50, or 100 mL of water per day. The treatments included three irrigation regimes, with three replicates of ten potted plants per treatment; experimental design corresponded to a completely randomized experiment with three replications, the experimental unit corresponded to ten strawberry plants.

This trial is in progress, for evaluation strawberry plants will be rated for the severity of Charcoal rot based on an ordinal scale of 1-5 at five, six, seven, and eight weeks after transplanting. Ratings will be assigned as follows: 1 = healthy, 2 = mild stunting, 3 = severe stunting and yellowing of leaves, 4 = severe stunting, and chlorotic and necrotic leaves, and 5 = dead.

The substrate moisture content will be determined three hours after the irrigation by inserting a soil moisture sensor in the root zone at the middle of each pot height. Water relations, in the form of Stem Water Potential (SWP) will be taken weekly starting at week four after transplanting. The evaluations will be performed at noon during a time of relatively high crop water demand (between 11:00 and 15:00 hours). Water relations (SWP, gs) will be measured in completely expanded trifoliate leaves located at the central portion of the plant. SWP will be measured using a pressure chamber; gs (mmol m–2 s–1) will be measured using a portable porometer.

All statistical analyses will be carried out using SigmaStat 3.1 (Systat Software Inc., San Jose, CA). All the evaluated parameters will be analyzed via a factorial analysis: cultivar and irrigation (3 x 3). The means will be separated according to Fisher's least significant difference test ($P < 0.05$). The correlations coefficients between the disease severity (AUDPC) and the physiological characteristics will be calculated. The trial will be repeated the following year. The same trial will be performed for Fusarium wilt.

Effect of Drought Stress on the Development of Fusarium wilt and Charcoal rot in the Field.

This objective will be carried out during the fall of 2023.

Results

Effect of Soil Inoculum Density on the Development of Fusarium wilt and Charcoal rot on Different Strawberry Cultivars

<u>Fusarium wilt</u>

In Davis, symptoms of Fusarium wilt were evident at the beginning of early June 2022, in the susceptible cultivar 'Monterey', with mean mortality of 68.8 in plants exposed to Level 3, 50% in Level 4, and 43.8 in Level 5. The disease developed to a different extent in the other susceptible cultivar 'UCD Mojo', with mean mortality of 12.5% only in plants exposed to Level 3. The resistant cultivar 'UCD Victor', was free of symptoms in all four repetitions when exposed to all the studied inoculum levels (Table 1, Figure 1).

TABLE 1

Mortality percentages of strawberry cvs 'UCD Victor', 'UCD Mojo' and 'Monterey', and F. oxysporum f. sp. fragariae inoculum densities (CFU/g) related to each experimental level in Davis, CA, 2022

Level	CFU/g		Mortality, % Cultivar 'UCD Victor'		'UCD Mojo'		'Monterey'	
1	31.3	a	0.0	n.s.	0.0	n.s.	0.0	a
2	87.5	ab	0.0		0.0		0.0	a
3	412.5	ab	0.0		12.5		68.8	c
4	562.5	ab	0.0		0.0		50.0	bc
5	843.8	b	0.0		0.0		43.8	b

Different letters indicate significant differences between within a column by Fisher LSD analysis (P < 0.05). ns = not significant.

At the end of this experiment in Davis, *F. oxysporum* f. sp. *fragariae* inoculum densities were divided into three, being the averages 31.3 (Level 1), 352.3 (Levels 2, 3, and 4), and 843.8 (Level 5). No statistical differences in the number of CFU/g of soil were observed between experimental levels 2, 3, and 4 (P > 0.05) (Table 1, Figure 1).

As expected, mortality ratings for susceptible cultivar 'Monterey', was higher at each level (Figure 1). 'Monterey' plants presented a low tolerance to *F. oxysporum* f. sp. *fragariae* inoculum levels of 412.5 CFU/g soil, at which more of the 40% of the plants were dead. However, different results were observed the susceptible cultivar 'UCD Mojo' showed a high tolerance to the disease (Figure 1).

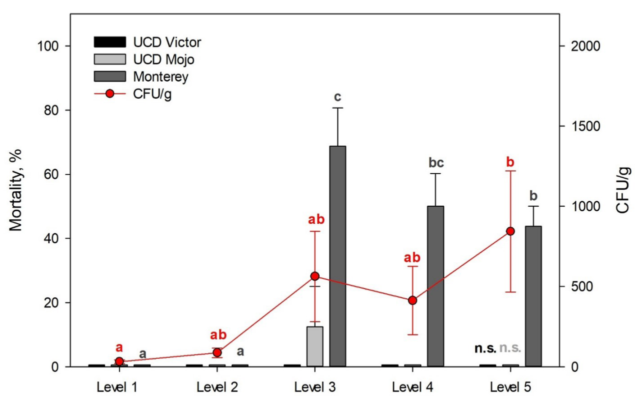

FIGURE 1

Mortality percentages of strawberry plants cvs. 'UCD Victor', 'UCD Mojo' and 'Monterey', and *F. oxysporum* f. sp. *fragariae* inoculum densities (CFU/g) related to initial inoculum levels in Davis, CA, 2022. Level 1, Level 2, Level 3, Level 4, and Level 5 correspond to planting holes initially inoculated with one liter of soil at 0, 100, 500, 1,000, and 2,000 CFU/g respectively. Different letters indicate significant differences in mortality percentage between initial inoculum level (grey) and CFU/g of soil determined at the end of the season (red) by Fisher LSD analysis (P < 0.05). Bars = standard error of the mean of four replicates.

Charcoal rot. In Davis, symptoms of Charcoal rot were evident at the end of May 2022, and continuing through the end of the experiment in July. Symptoms were observed in all cultivars, from Level 2 to Level 5 in 'Fronteras' and 'UCD Victor', and from Level 3 to Level 5 in 'UCD Mojo'. (Table 2, Figure 2).

A significant effect (P < 0.001) of the inoculum levels and the cultivars was observed in the development of Charcoal rot mortality.

Cultivars 'Fronteras' and 'UCD Victor', considered "moderately susceptible" to *M. phaseolina*, presented the similar percentage of mortality at each level of inoculum. Average mortality was 37.5% at Level 2 (119.8 CFU/g), 53.3% at Level 3 (833.3 CFU/g), 65.7% at Level 4 (4,849 CFU/g), and 81.3% at Level 5 (13458.4 CFU/g) (Table 2, Figure 2).

Mortality rates were lower in cultivar 'UCD Mojo', considered "moderately resistant" to *M. phaseolina*. Average mortality of 6.3% was observed in plants exposed to 833.3 CFU/g, 25% in plants exposed to 4,849 CFU/g, and 50% in plants exposed to 11,3458.4 CFU/g (Table 2, Figure 2).

TABLE 2

Mortality percentages of strawberry cvs. 'UCD Mojo', 'Fronteras' and 'UCD Victor', and M. phaseolina inoculum densities (CFU/g) related to each experimental level in Davis, CA, 2022

Level	CFU/g		Mortality, % Cultivar 'UCD Mojo'		'Fronteras'		'UCD Victor'	
1	20.8	a	0.0	a	0.0	a	0.0	a
2	119.8	b	0.0	a	37.5	ab	37.5	ab
3	833.3	c	6.3	a	56.3	b	56.3	bc
4	4,849.0	d	25.0	ab	62.5	b	68.8	bc
5	13,458.4	e	50.0	b	75.0	b	87.5	c

Different letters indicate significant differences between within a column by Fisher LSD analysis (P < 0.05).

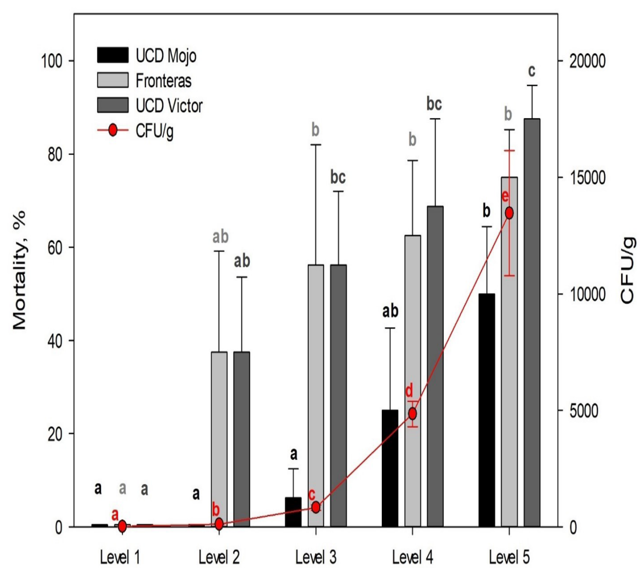

FIGURE 2

Mortality percentages of strawberry plants cv. 'UCD Mojo', 'Fronteras' and 'UCD Victor', and *M. phaseolina* inoculum densities (CFU/g) related to initial inoculum levels and assayed inoculum levels in Davis, CA, 2022. Level 1, Level 2, Level 3, Level 4, and Level 5 corresponds to planting holes initially inoculated with one liter of soil at 0, 25, 100, 1,000, and 10,000 CFU/g respectively. Different letters indicate significant differences in mortality percentage between initial inoculum level (grey) and CFU/g of soil determined at the end of the season (red) by LSD analysis (P < 0.05). Bars = standard error of the mean of four replicates.

Effect of Drought Stress on the Development of Fusarium wilt and Charcoal rot Under Greenhouse Conditions

This objective is currently in progress at the UC Davis greenhouse, plants have been planted in 1-L pots and will be inoculated in mid-August, 2022. After the pathogen inoculation, plants will be watered daily with rates of 25, 50, or 100 ml, of water per day.

Effect of Drought Stress on the Development of Fusarium wilt and Charcoal rot in the Field

For this objective, the soil inoculation and planting of the strawberry plants will be carried out during the fall of 2022.

Discussion

Soil inoculum density of *F. oxysporum* f. sp. *fragariae* was found to cause mortality in the known susceptible cultivar 'Monterey' but not in 'UCD Mojo' and 'UCD Victor'. We had similar results in our previous studies, where susceptible cultivars ('Albion' and 'Monterey') responded to diverse inoculum levels of *F. oxysporum* f. sp. *fragariae*. A general threshold for Fusarium wilt development was established at inoculum levels greater than 100 CFU/g soil of *F. oxysporum* f. sp. *fragariae*. This information will help growers to determine whether it is safe to grow certain cultivars based on the Fusarium wilt pathogen inoculum level.

Soil inoculum density of *M. phaseolina* was also found causal mortality in cultivars with moderate susceptibility ('UCD Victor', and 'Fronteras') and in cultivars with moderate resistance to Charcoal rot ('UCD Mojo'). However, the new cultivar 'UCD Mojo' showed the lowest mortality in all the inoculum levels in these field trials.

In conclusion, soil inoculum density plays an important role in terms of the mortality of the plant. However, other factors such as soil type, weather conditions and other stress factors also contribute to plant mortality.

Knowledge of threshold levels for the development of Fusarium wilt and Charcoal rot will also allow strawberry growers to make more informed decisions concerning disease management strategies to reduce losses, such as pre-plant fumigation and/or cultural practices to suppress or reduce pathogen populations in soil.

Similar results were observed the trial in Santa Paula (2021). Charcoal rot symptoms were observed in all cultivars, from Level 1 to Level 5 in 'Fronteras' and 'UCD Victor', and from Level 2 to Level 5 in 'UCD Mojo'. (Table 3, Figure 3).

Cultivars 'UCD Victor' and 'Fronteras', presented a similar percentage of mortality (Table 3). Average mortality was 12.3% at Level 1 (62.5 CFU/g), 40.7% at Level 2 (291.7 CFU/g), 61.1% at Level 3 (447.9 CFU/g), 96.7% at Level 4 (8,989.6 CFU/g), and 100% at Level 5 (19,072.9 CFU/g). No significant differences in mortality rates were observed when these plants were exposed to inoculum levels between 8,989.6 and 19,072.9 CFU/g. (Table 3, Figure 3). Mortality rates were lower in cultivar 'UCD Mojo', Average mortality was 18.8% observed in plants exposed from 291.7 to 447.9 CFU/g, and 62.5% in plants exposed from 8,989.6 to 19,072.9 CFU/g (Table 3, Figure 3).

TABLE 3

Mortality percentages of strawberry cvs. 'UCD Mojo', 'Fronteras' and 'UCD Victor', and *M. phaseolina* inoculum densities (CFU/g) related to each experimental level in Santa Paula, CA, 2022

Level	CFU/g		Mortality, % Cultivar 'UCD Mojo'		'Fronteras'		'UCD Victor'	
1	62.5	a	0.0	a	18.8	a	6.3	a
2	291.7	b	18.8	a	43.8	b	37.5	b
3	447.9	b	18.8	a	68.8	c	53.3	c
4	8,989.6	c	62.5	b	93.8	d	100.0	d
5	19,072.9	d	62.5	b	100.0	d	100.0	d

Different letters indicate significant differences between within a column by Fisher LSD analysis (P < 0.05).

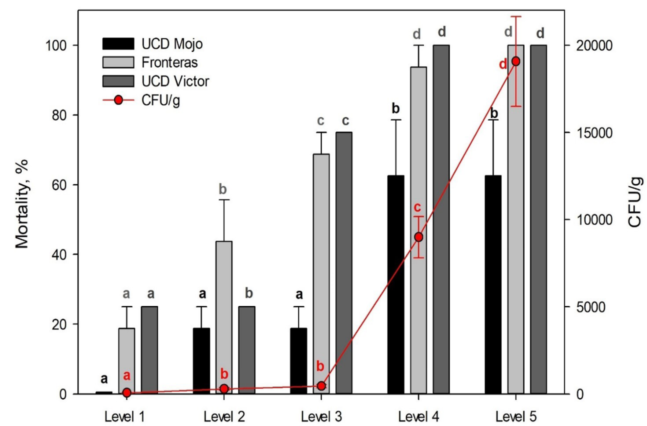

FIGURE 3

Mortality percentages of strawberry plants cv. 'UCD Mojo', 'Fronteras' and 'UCD Victor', and *M. phaseolina* inoculum densities (CFU/g) related to initial inoculum levels and assayed inoculum levels in Santa Paula, CA, 2022. Level 1, Level 2, Level 3, Level 4, and Level 5 corresponds to holes initially inoculated with one liter of soil at 0, 25, 100, 1,000, and 10,000 CFU/g respectively. Different letters indicate significant differences in mortality percentage between initial inoculum level (grey) and CFU/g of soil determined at the end of the season (red) by LSD analysis (P < 0.05). Bars = standard error of the mean of four replicates.

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