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# Year 5: Evaluating Strawberry Cultivars for Resistance to Verticillium wilt



Co-Investigators
Dr. Gerald Holmes
Director, Strawberry Center
California Polytechnic State University, San Luis Obispo (805) 756-2120
gjholmes@calpoly.edu



Dr. Seyed Mojtaba Mansouripour
Postdoctoral Researcher
California Polytechnic State University, San Luis Obispo (805) 756-6157
smansour@calpoly.edu



Dr. Shashika Hewavitharana Assistant professor, Strawberry Center California Polytechnic State University, San Luis Obispo (805) 756-2120 shewavit@calpoly.edu



**Dr. Bo Liu**Assistant Professor, BioResource & Agricultural Engineering Department California Polytechnic State University, San Luis Obispo, CA (805) 756-2384
Bliu17@calpoly.edu

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#### SUMMARY

The reduction in efficacious fumigants and increased regulations surrounding their use has exacerbated soilborne diseases in strawberry. When methyl bromide was available before 2016, focus for strawberry breeding for soilborne disease resistance was a lower priority than other horticultural traits. This practice significantly hindered the progression of breading for disease resistance. This can be remedied by a focus on breeding for disease resistance, and a firm understanding of how strawberry cultivars will perform where high soil inoculum levels exist. We evaluated 65 strawberry cultivars and elite selections for susceptibility to Verticillium wilt in field soil naturally infested with *Verticillium dahliae* over the course of the 2019-2020 season. Plant mortality was assessed over time in both inoculated and non-inoculated plots. Genotypes varied widely in their response to the disease, from zero to 61.9% plant mortality, with an average mortality of 14.3% for all genotypes and 17% for 28 cultivars common to the 2019 and 2020 experiments. This project directly addresses these high priority research areas identified by the California Strawberry Commission for the central coast region: (1) farming without fumigants; (2) control of soilborne diseases; and (5) breeding for disease resistance.

## Introduction

The use of resistant cultivars is the most widely adopted and effective integrated pest management strategy in all of agriculture. However, prior to 2016, preplant fumigation with methyl bromide and chloropicrin was so efficacious at controlling soilborne diseases of strawberries that it significantly reduced the practical importance of host plant resistance. With the phase-out of methyl bromide and rising environmental and worker safety concerns over the use of all fumigants, the significance of plant resistance is now being reemphasized. To facilitate effective usage of host plant resistance to manage soilborne diseases, it is important to demonstrate to growers how current and future genotypes will perform in field soils naturally infested with soilborne pathogens. Verticillium wilt of strawberry is caused by Verticillium dahliae which is a pathogen to over 400 hosts including common rotational crops with strawberry such as lettuce. Based on Shaw et al., (2010), resistance to Verticillium wilt has been a selection criterion in the UC breeding program for over twenty years. Therefore, significant gains in basal levels of resistance within the UC breeding population have been achieved (Shaw et al., 2010). Since these studies used root dip inoculations, it is yet to be established how currently available cultivars will perform in the presence of natural soilborne inoculum of microsclerotia (Gordon et al., 2012). In addition, due to significant year x genotype interactions, multi-site, multi-year cultivar evaluations are warranted. The field location selected for this study has high levels of natural soilborne V. dahliae inoculum. We have preserved this site for the purpose of evaluating control measures against Verticillium wilt. The objective of this study was to characterize commercial strawberry cultivars and elite breeding lines for their susceptibility to Verticillium wilt under field conditions in naturally infested field soil.

#### MATERIALS AND METHODS

A replicated field trial was established to evaluate 65 cultivars and elite selections for resistance to Verticillium wilt caused by *V. dahliae*. Strawberry germplasm was selected from six public and private breeding programs: University of California Davis, University of Florida, Driscoll's, Plant Sciences, Lassen Canyon Nursery and California Berry Cultivars. For the purpose of comparing year-to-year variation, there were 28 cultivars common between this year and last year.

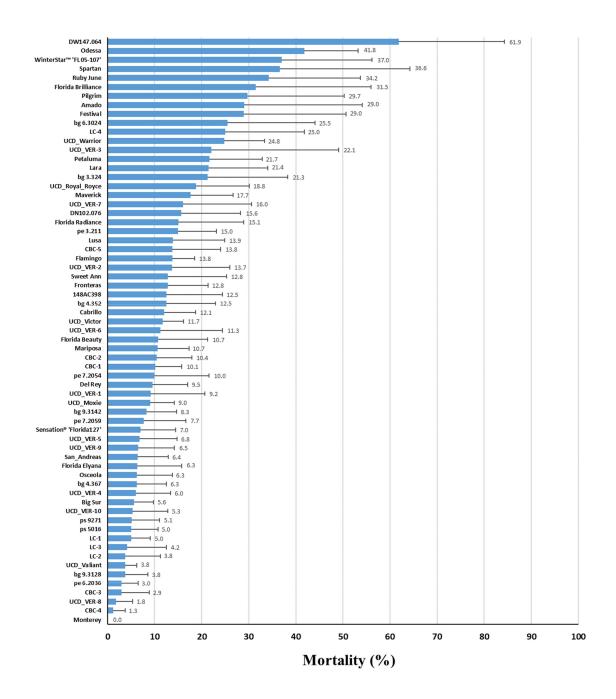
On October 23, 2019, bare-root strawberry transplants were planted in field 25, block 8 on the Cal Poly San Luis Obispo campus. This field had a history of Verticillium wilt and was naturally infested due to decades of cropping to Verticillium wilt susceptible crops such as lettuce and tomato. The trial consisted of 20-plant plots replicated four times, with a fifth control replicate planted in an area of the field that was fumigated with Ally 33 (67% AITC + 33% chloropicrin at 55 gal/A) in the fall of 2019. Host resistance was assessed by recording disease incidence (plant mortality) in each plot. A plant was considered dead when 100% of the foliage was brown and dried up. Plant mortality was assessed every two weeks; the last assessment occurred on August 3, 2020.

Symptomatic plants were sampled during evaluations throughout the experiment to determine which pathogens were present. Presence of a pathogen was confirmed by plating pieces of symptomatic petioles on NP-10 medium (Mihail, 1992). Additionally, the internal crown tissue was plated on acidified potato dextrose agar media (APDA) and pimarcin + ampicillin + rifampicin + pentachloronitrobenzene agar (PARP) (Mihail, 1992). NP-10 was used to isolate *Verticillium* spp (Kabir et al., 2004). APDA was used to detect *Macrophomina phaseolina* and *Fusarium* spp. PARP media was used to isolate Phytophthora spp. If *Fusarium* spp. was isolated, the crown tissue was tested for pathogenic *Fusarium oxsporum* f. sp. *fragariae* using the isothermal recombinase polymerase amplification method (Burkhardt et al., 2019). If any other pathogen except *Verticillium* spp. was isolated, the baseline number of plants per plot was adjusted accordingly. Aerial photographs were taken at approximately two-week intervals using an unmanned aerial vehicle (UAV) drone to track disease progress.

#### RESULTS

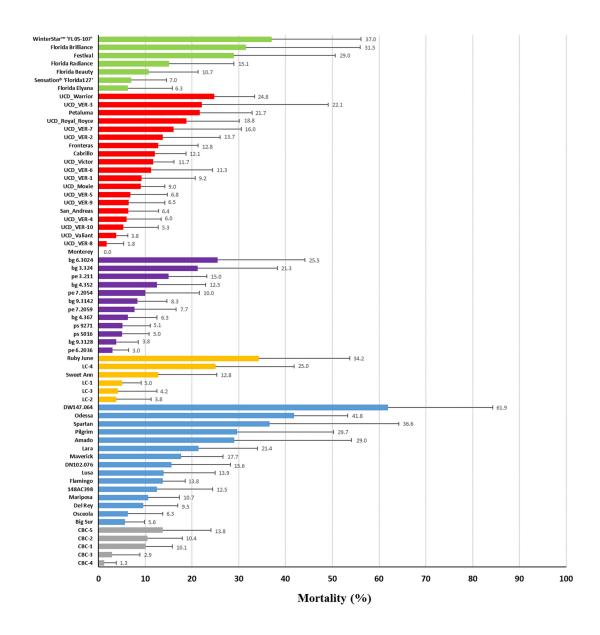
The first wilt symptoms due to infection by *V. dahliae* were observed in early May, roughly 200 days after planting. A wide range of susceptibility was observed among all genotypes (Figure 1). There was a wide spectrum of genotype susceptibility within each of the six breeding programs with the exception of California Berry Cultivars whose five genotypes showed an average of 7.7% mortality (Figure 2). The average mortality across all genotypes was 14.3%, compared to 12.5% in 2019 and 17.0% (2020) compared to 15.6% (2019) for the 28 cultivars common to both years. 'Odessa' (41.8%), and 'DW147.064' (61.9%) were the most susceptible genotypes as measured by plant mortality on August 3, 2020. 'Monterey' was the most resistant cultivar to Verticillium wilt, with 0% mortality by August 3, 2020. The most resistant genotypes were 'CBC-4' (1.3% mortality), and 'UCD\_VER-8' (1.8% mortality).

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*Figure 1.* Average percent plant mortality due to Verticillium wilt as of August 2020. Error bars represent the standard error of the mean.

Sampling of symptomatic plants resulted in 43 individual plant samples. Of these 43 samples, seven (16%) were positive for *Verticillium* spp., four (9%) were positive for *M. phaseolina*, three (7%) were positive for *Pestalotia*-like (pathogenicity not confirmed), one (2%) was positive for *Phytophthora* spp., and for 28 (65%) samples, no pathogen was recovered. We speculate that the 28 samples where no pathogen was recovered may have been positive for Verticillium, but because of the advanced state of disease in these samples, the pathogen was not recovered.



*Figure 2.* Average percent plant mortality due to Verticillium wilt as of August 2020, sorted by color representing different breeding program. Error bars represent the standard error of the mean.

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#### Discussion

The distribution of genotype susceptibility was continuous between zero and 61.9% plant mortality. Entries of all breeding programs included a spectrum of tolerant and susceptible germplasm to Verticillium wilt. Last year's experiment showed 'Monterey" as one of the most susceptible genotypes at 25.7% mortality whereas this year it was the most resistant genotype in the entire experiment. We confirmed that the plant material was not mislabeled and are unable to explain the reason for this difference. No other outliers of this sort were detected in the dataset. Findings of this study are important to the growers to select cultivars to manage Verticillium wilt, and to the breeding programs for validating resistance to Verticillium wilt resistance under field conditions with natural inoculum.

#### **ACKNOWLEDGMENTS**

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