

Final Report to California Leafy Greens Research Program 2016-2017

Project Title: Comparative Fitness of Race 1 and Race 2 Strains of *Verticillium dahliae* Affecting Lettuce

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Abstract

Verticillium dahliae Race 2 is a serious threat to lettuce production in Coastal California. However, Race 2 isolates have not been detected on lettuce in the Salinas Valley despite the proximity of Pajaro Valley where Race 2 exists, and their widespread presence on spinach seed and other crops. Race 2 is expected to expand and become dominant following the deployment of lettuce cultivars with Race 1 resistance, but little information is available to understand the speed at which this establishment and dominance might occur. The objective of this research is to compare the fitness of Race 1 and Race 2 *V. dahliae* strains to understand why Race 2 has not yet become established in the Salinas Valley. To date, several attempts to produce the microsclerotia inoculum that is required in this study to accurately represent the disease cycle have not been successful for all isolates. We are currently performing several experiments that include specialized culture media, re-isolating from infected plants, and directly using infested plants in order to complete this funded project.

Objectives

- A. Determine if Race 2 isolates of *V. dahliae* have reduced survivability in field soil compared to Race 1
- B. Evaluate pathogenic aggressiveness of Race 1 and Race 2 strains
- C. Compare microsclerotia production in infested tissue between Races 1 and 2

Procedures

To first determine the inoculum levels to use in subsequent experiments for Objectives B and C, microsclerotia-sand inoculum was produced by pouring a conidial suspension of *V. dahliae* isolate Ls16 into Petri plates containing sterilized fine sand. Plates were incubated and then dried, and the number of colony forming units of *V. dahliae* was assessed by sprinkling inoculum on plates containing NP-10. Soil for the experiment was collected from Field C at the USDA-ARS Station in Salinas where repeated attempts have been made to establish a race 2-infested soil. In Trial 1, soil was steamed and amended with the microsclerotia-sand inoculum to final concentrations of 0, 5, 10, 20, 35, 55, 80, 110, 150 microsclerotia g⁻¹ field soil. Plant introduction (PI) 251246, an early bolting lettuce highly susceptible to *Verticillium* wilt, was transplanted into 16 fl. oz. Styrofoam cups containing inoculated soil. Trial 2 was a two-factor factorial consisting of four inoculum levels (0, 100, 250, 400 microsclerotia g⁻¹ field soil) and two hosts (PI 251246 and eggplant cv. Orient Express). Soil from the same field was autoclaved, amended with the microsclerotia-sand, and placed in Styrofoam cups. Then, the plants were directly seeded into the inoculated soil. At flowering, plants were removed from cups and washed of soil, and the taproot and lower stem were bisected and assessed for vascular discoloration on a 0 to 5 scale.

Next, population genetic (microsatellite and DNA sequence) and race data that was collected in previous CLGRP-funded research in the Subbarao lab was used to select the *V. dahliae* isolates to be used in this study. Four race 1-race 2 isolate pairs that were otherwise very similar according to the genetic data, in this case having identical alleles at 13 microsatellite loci, were selected (Table 1). Upon receiving these isolates at UCR, an alternative method of producing microsclerotia was tried in which isolates are grown on standard culture media on the lab bench at room temperature, dried, then mixed with sand.

Table 1. *V. dahliae* isolates selected for race fitness evaluation.

| Isolate | Original Host | Pair | Race | Microsatellite Haplotype ¹ | ITS Sequence Haplotype |
|---------|---------------|------|------|---------------------------------------|------------------------|
| Gh 1016 | Cotton | A | 1 | 448 | 1 |
| Gh 1011 | Cotton | A | 2 | 448 | 1 |
| So 929 | Spinach | B | 1 | 351 | 6 |
| So 2381 | Spinach | B | 1 | 351 | n/a |
| So 941 | Spinach | B | 2 | 351 | 15 |
| So 2581 | Spinach | B | 2 | 351 | n/a |
| Gh 1015 | Cotton | C | 1 | 348 | 6 |
| So 2578 | Spinach | C | 2 | 348 | n/a |

¹ Haplotype = Isolates with the same haplotype possess identical alleles or sequence for the given genetic marker.

Because an insufficient amount of microsclerotia was produced with this method, a pilot study was conducted to determine growth conditions that increase microsclerotia production by all isolates. The eight selected isolates (Table 1) plus Ls16 were incubated in: 1) a cold room at 20°C in the dark; 2) an incubator at 25°C with 24 hr of fluorescent light; 3) the lab bench at 23°C with about 8 hr of fluorescent light on 12 replicate plates. Colony diameter was measured and the percent area of the mycelial colony producing microsclerotia was visually estimated weekly for six weeks.

Results and Discussion

Verticillium wilt incidence was low in Trial 1, and there were no significant differences among treatments (Figure 1). No Verticillium wilt developed in Trial 2.

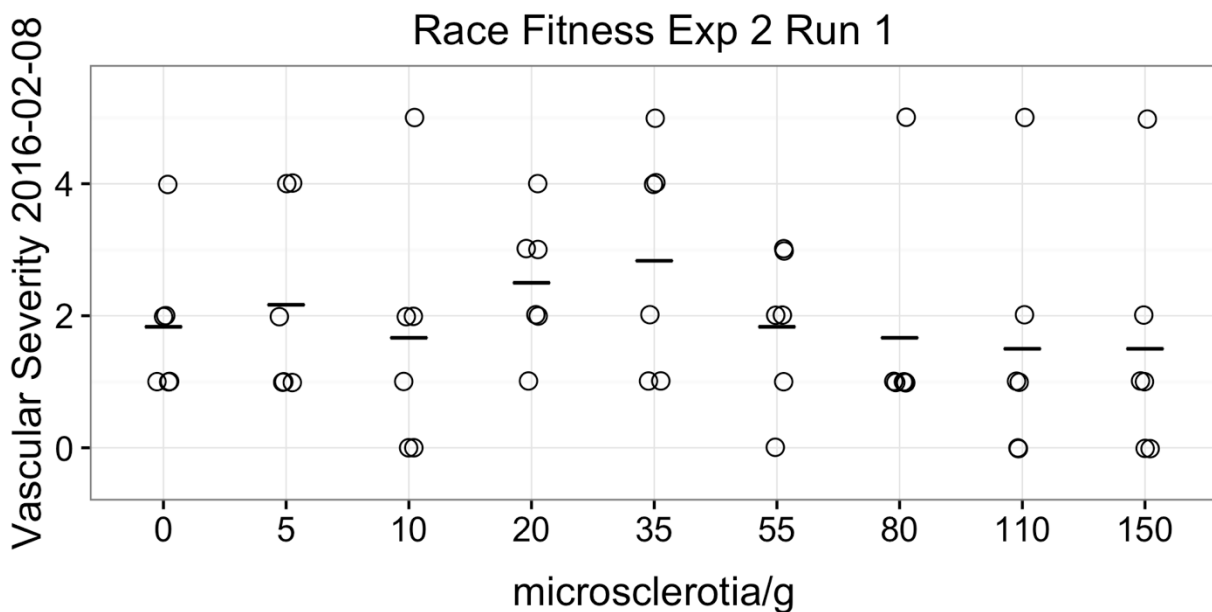


Figure 1. Trial 1: Vascular discoloration of PI 251246 on a 0 to 5 scale where 0 = healthy and 5 = dead as influenced by rate of inoculation by microsclerotia-sand inoculum.

In the initial attempt to culture isolates for microsclerotia, only three of the eight isolates had produced a sufficient amount of microsclerotia after approximately six weeks of growth. In the pilot study, microsclerotia production was greatest in the cold room compared to the incubator or lab bench (Figure 2). However, two isolates (Ls16 and So2581) did not produce any microsclerotia at all, and an additional three isolates (Gh1015, Gh1016, So941) produced an insufficient amount of microsclerotia for inoculation. The lack of microsclerotia production by Ls16 in this experiment might explain the lack of disease in Trials 1 and 2.

We are currently addressing the lack of success at microsclerotia production in three ways. First, the inoculum used for Objective A will be switched from culture-based microsclerotia-sand to more natural inoculum produced in planta. Lettuce cv. Salinas was seeded into 128 well germination trays, and is being inoculated three times on a four day interval with a suspension of 1×10^7 conidia mL^{-1} of one of the eight isolates. After the final inoculation, seedlings will be transplanted to 0.7 gal, 6 in. diameter pots containing a 1:1 sand:organic matter mixture. Shortly

after maturity, plants will be removed, cleaned of loose soil, chopped into small pieces, laid to dry, then finely ground. Ground, infested lettuce tissue will be amended to the USDA-ARS Salinas Field C soil at the same concentration for each isolate, and placed in 6 in. diameter pots. Two lettuce plants will be inoculated for each of the six replicate pots in the experiment. The concentration of viable microsclerotia will be assessed every four weeks by the standard soil impaction plating technique. Although this method of producing inoculum requires long waiting periods, it more closely simulates the actual disease cycle of *Verticillium* wilt and is therefore likely to provide more accurate information for practical situations. For example, a study on the soilborne pathogen *Macrophomina phaseolina*, which produces microsclerotia like *V. dahliae*, reported that microsclerotia produced on infested tissue survived in soil longer than culture-produced microsclerotia.

Second, we will try to produce microsclerotia by culturing isolates on an alkaline-modified sodium polypectate liquid medium and a modified basal liquid medium media, which were shown in a recent publication to maximize microsclerotia production of *V. dahliae* isolates from olive, especially one that did not previously produce microsclerotia in artificial media. Third, we will attempt to induce microsclerotia production by performing a “pass-through” experiment, a common tactic in plant pathology to regain the original virulence or other trait lost by an isolate during storage and artificial cultivation. PI 251246 and eggplant cv. Orient Express were germinated in 36 well (2 in. x 2 in.) trays, and are in the process of being inoculated with conidial suspensions of one of the eight isolates to be used in this study. The pathogen will be isolated from plants following disease development, and these “passed” isolates will be cultured on the specialized modified media to produce inoculum for Objective B and C experiments.

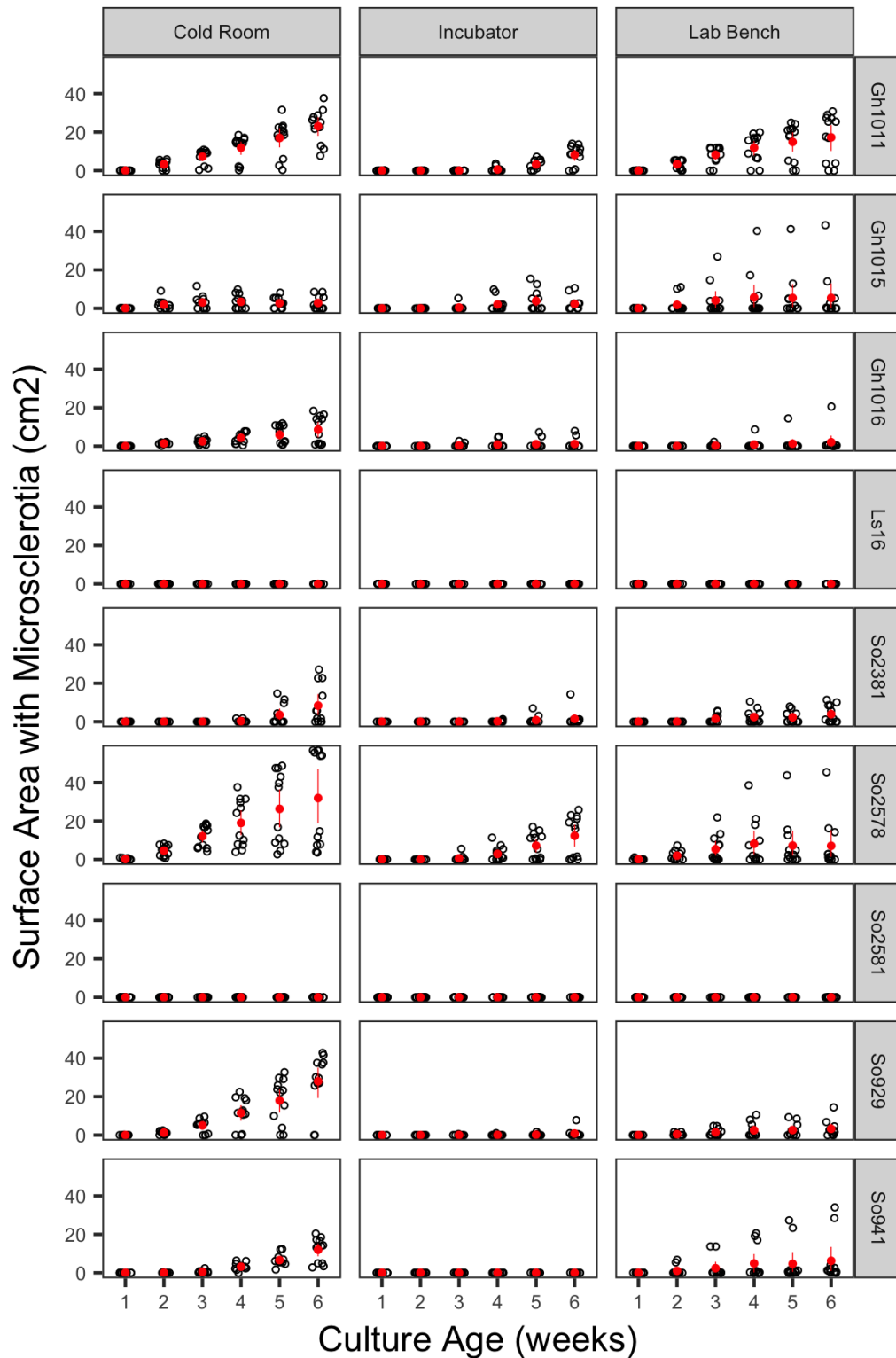


Figure 2. Surface area of *V. dahliae* with microsclerotia (cm²) as influenced by incubation in: **Cold Room**, 20°C, no light; **Incubator**, 25°C, 24 hr light; **Lab Bench**, 23°C, 8 hr light. **Hollow black circles**, data points for each replicate plate; **red points and bars**, mean and 95% confidence limits.