

## Monitoring the population of the lettuce *Fusarium* wilt pathogen in California

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### Objectives

1. Collect FOL samples representing the population in the Imperial Valley and Huron area and areas of concern in the Salinas Valley  

Deliverable: list of samples along with associated information (date of collection, anonymized location, lettuce type and cultivar)
2. Characterize race, phenotypic, and genetic variation of the sampled isolates
  - a. Deliverable: race, vegetative compatibility group, and DNA sequence type of the collected FOL samples

### Abstract

*Fusarium* wilt of lettuce, caused by the soil inhabiting fungus *Fusarium oxysporum* f. sp. *lactucae* (FOL), can cause significant losses and has been a recurring pest problem in the fall to spring production regions of California. In the past two to three years, however, concern over *Fusarium* wilt has increased following an overall expansion in the prevalence and severity of the disease. Additionally, anecdotal observations and preliminary experiments suggest a non-race 1 strain is present at multiple locations. Little recent information is available on the FOL population in these regions, therefore an updated assessment is warranted. The objectives of this proposal are to: 1) collect a recent and representative sample of FOL from the Imperial Valley, the Huron area, and areas of new concern in the Salinas Valley; and 2) characterize the sample for pathogenic race, vegetative compatibility group, and DNA sequence type. A total of 192 *F. oxysporum* isolates have been collected from 33 locations to date. A total of 69% and 15% of the isolates were obtained from Monterey County and the Santa Maria Valley, respectively, suggesting that *Fusarium* wilt has recently become an important issue on the Central Coast relative to other production areas in California. To date, 14 isolates have been confirmed as FOL in pathogenicity screens, and 11 of these have been classified as members of the FOL race 1/race 4 clade based on DNA sequencing at five loci. One isolate was genetically distinct from the

FOL race 1/race 4 group and was found to be not pathogenic to lettuce. Pathogenicity screening, race typing, and other characterizations of remaining isolates is ongoing.

## **Procedures, Results, and Discussion**

### Objective 1 - Collect FOL samples representing the population in the Imperial Valley and Huron area, and areas of concern in the Salinas Valley

Fields sampled in the survey were arbitrarily selected based on responses to sample requests and observations of disease activity. Plants exhibiting symptoms of Fusarium wilt were arbitrarily selected and collected from each field. Plants were shipped or transported to the lab, and were subjected to standard plant pathology techniques to isolate FOL from symptomatic taproot tissue onto Komada's semi-selective culture medium. To date, a total of 192 *Fusarium oxysporum* isolates from 33 fields have been collected and stored in the lab (Table 1). A total of 65% and 15% of the isolates were obtained from iceberg and Romaine types, respectively (Table 2). A total of 69% and 15% of the isolates were obtained from Monterey County and the Santa Maria Valley, respectively. The level of interest from these Central Coast regions relative to the desert and Central Valley was unexpected and likely reflects that Fusarium wilt has rapidly emerged to become prevalent on the Central Coast.

To confirm that the isolates are the true FOL pathogen and not a non-pathogenic strain of *F. oxysporum*, pathogenicity of the isolates is being assessed. Briefly, seedlings of highly susceptible iceberg lettuce cultivar 'Grizzly' at the 2-3 leaf stage will be gently removed from the germination tray, the roots trimmed, and dipped in a suspension containing  $1 \times 10^7$  spores mL<sup>-1</sup> of FOL for 10 minutes. Inoculated seedlings will be planted into 3.5" pots at three or four plants per pot with sterile potting mix and maintained in a greenhouse with a day/night cycle of 91°F/73°F. Pots will be arranged in a randomized complete block with 4 replications. After 14 to 28 days, exterior symptoms of Fusarium wilt severity will be assessed on a 1 to 4 scale where 1 = healthy and 4 = dead. The experiment will be conducted twice. Approximately 5 pathogenic FOL isolates per field will be retained for further analysis.

To date, 15 isolates have been evaluated in the pathogenicity tests. A total of 14 of these were identified as FOL in this assay (Figure 1). The remaining isolate caused very mild symptoms, suggesting it is not FOL. Screening of isolates not yet tested is ongoing.

### Objective 2 - Characterize race, phenotypic, and genetic variation of the sampled isolates

Pathogen FOL isolates will be subjected to race typing. The procedure will be identical to that described above for the pathogenicity testing, except each isolate will be evaluated against the race differential set (Table 3). The experiment will be conducted twice. Data will be analyzed with the GLIMMIX procedure in SAS 9.4 using the multinomial distribution for ordinal data.

In the course of the population survey (Objective 4), we received samples from locations in which reactions of cultivars to Fusarium wilt were inconsistent of FOL race 1. The criteria for suspecting a non-race 1 strain are either a known susceptible cultivar exhibiting tolerance, a known tolerant cultivar exhibiting susceptibility, or both. To date, we have obtained isolates from 11 locations in which the potential race variant is present, 8 in Monterey County and 3 in the Santa Maria Valley. To date, race typing has been initiated on 8 isolates, but only for two of

the four differential cultivars due to unavailability of Romabella and the need to optimize conditions to germinate Banchu Red Fire. Although there is variation among these 8 isolates in Fusarium wilt severity on Costa Rica #4, the reactions on Costa Rica #4 and Patriot are consistent with race 1. However, because results from Dr. Richardson indicate that Banchu Red Fire is required to differentiate the potential variant from race 1 strains, our results for the 8 isolates are currently inconclusive.

Isolates will then be assigned to VCGs using methods described by Correll et al. (1987) and Paugh and Gordon (2020). Briefly, nitrogen non-utilizing (*nit*) mutants will be obtained for each isolate by culturing on minimal media amended with chlorate. Each isolate will be paired on minimal media with known tester strains, and the reaction between the isolates and testers will be assessed after 14 days of growth. Reactions will be scored on a 0 to 4 scale where 0 = no reaction, 1 = weakly positive reaction, and 4 = fully compatible reaction. Isolates will be assigned to a VCG based on a minimum score of 1, and will be assigned to a VCG subgroup based on a minimum score of 4. The experiment will be conducted with two replicate mutants and at least two replicate plates.

Genetic variation among a selection of FOL isolates will be assessed by extracting DNA, then amplifying and sequencing the translation elongation factor 1-alpha (TEF-1a) region and four anonymous loci under development by Dr. Martin for characterizing *F. oxysporum*. To date, 12 isolates have been sequenced at these loci, and 11 were found to be nearly 100% identical to all FOL race 1 and race 4 reference strains (data not shown). The remaining isolate was identified as distinct from the FOL race 1/race 4 group, and is the same isolate that was identified as not pathogenic to lettuce in the pathogenicity survey in Objective 1.

**Table 1.** Origin information of 33 sites sampled for *Fusarium oxysporum* f. sp. *lactucae*.

Date Collected	Region	County	Lettuce Type	Cultivar	# of isolates
2021-10-13		Fresno	iceberg	-	3
2021-10-13		Fresno	iceberg	-	12
2019-12-12		Imperial	iceberg	SV4204LD	5
2021-12-11		Imperial	iceberg	Slot Machine	12
2022-07-06	-	Monterey	-	-	4
2022-07-21	-	Monterey	iceberg	Steamboat	3
2022-08-04*	-	Monterey	iceberg	San Miguel	3
2021-09-24	North County	Monterey	romaine	Salvius	3
2022-07-29	North County	Monterey	iceberg	Spyglass	3
2021-09-30	North County	Monterey	butterhead	-	2
2022-08-12	North County	Monterey	iceberg	San Miguel	2
2021-10-13	North County	Monterey	iceberg	n/a	2
			differential	Pat., Red F.	8
2021-09-24	North County	Monterey	iceberg	-	10
2021-08-31	North County	Monterey	romaine	Solid King	7
2021-09-16*	North County	Monterey	iceberg	Tiber II	3
			differential	Banchu Red Fire	4
			iceberg	Paraiso	4
2022-09-17*	North County	Monterey	iceberg	Hercules	1
2021-09-16*	North County	Monterey	iceberg	San Miguel	2
			iceberg	Hercules	5
2021-09-16*	North County	Monterey	iceberg	-	1
2022-06-13*	North County	Monterey	iceberg	Hercules	3
			iceberg	San Miguel	2
			iceberg	Primo	2
2022-07-28*	North County	Monterey	iceberg	San Miguel	4
2022-07-29	North County	Monterey	differential	Banchu Red Fire	3
			iceberg	Primo	2
2022-05-12	North County	Monterey	iceberg	Reliant	5
2022-08-12	North County	Monterey	iceberg	Blackhorse	4
2021-08-03	Mid County	Monterey	romaine	-	10
2022-08-18	Mid County	Monterey	iceberg	Newcastle	3
			iceberg	Salute	3
2021-10-08	Mid County	Monterey	red leaf	-	10
2021-09-02	Mid County	Monterey	romaine	-	5
2022-06-24	Mid County	Monterey	iceberg	Tiber	3
2022-06-10*	Mid County	Monterey	iceberg	Outfitter	3
2021-09-02	South County	Monterey	romaine	-	3
2022-08-18	Santa Maria Valley	Santa Barbara	red leaf	Ruby Sky	3
2022-08-18	Santa Maria Valley	Santa Barbara	iceberg	Telluride	2
2021-10-14*	Santa Maria Valley	Santa Barbara	-	-	6
(2022-07-07)			iceberg	San Miguel	5
			iceberg	Paraiso	1
2022-06-03*	Santa Maria Valley	Santa Barbara	iceberg	Telluride	5
2022-08-12*	Santa Maria Valley	Santa Barbara	iceberg	Journey	6

\* = anecdotal observations suggest a non-race 1 strain is present at this location; - = not available; differential = cultivar(s) from the Fusarium wilt race differential set planted as indicators in small demonstration plots

**Table 2.** Summary of *Fusarium oxysporum* f. sp. *lactucae* isolates collected to date.

County	Region	# of Fields	Number of Isolates				Total
			Iceberg	Romaine	Other <sup>1</sup>	Diffs <sup>2</sup>	
Fresno	Central Valley	2	15				15
Imperial		2	17				17
Monterey	North County	16	55	10	2	15	82
Monterey	Mid County	6	12	15	10		37
Monterey	South County	1		3			3
Monterey	n/a	3	6		4		10
Santa Barbara	Santa Maria	5	19		9		28
TOTAL		33	124	28	25	15	192

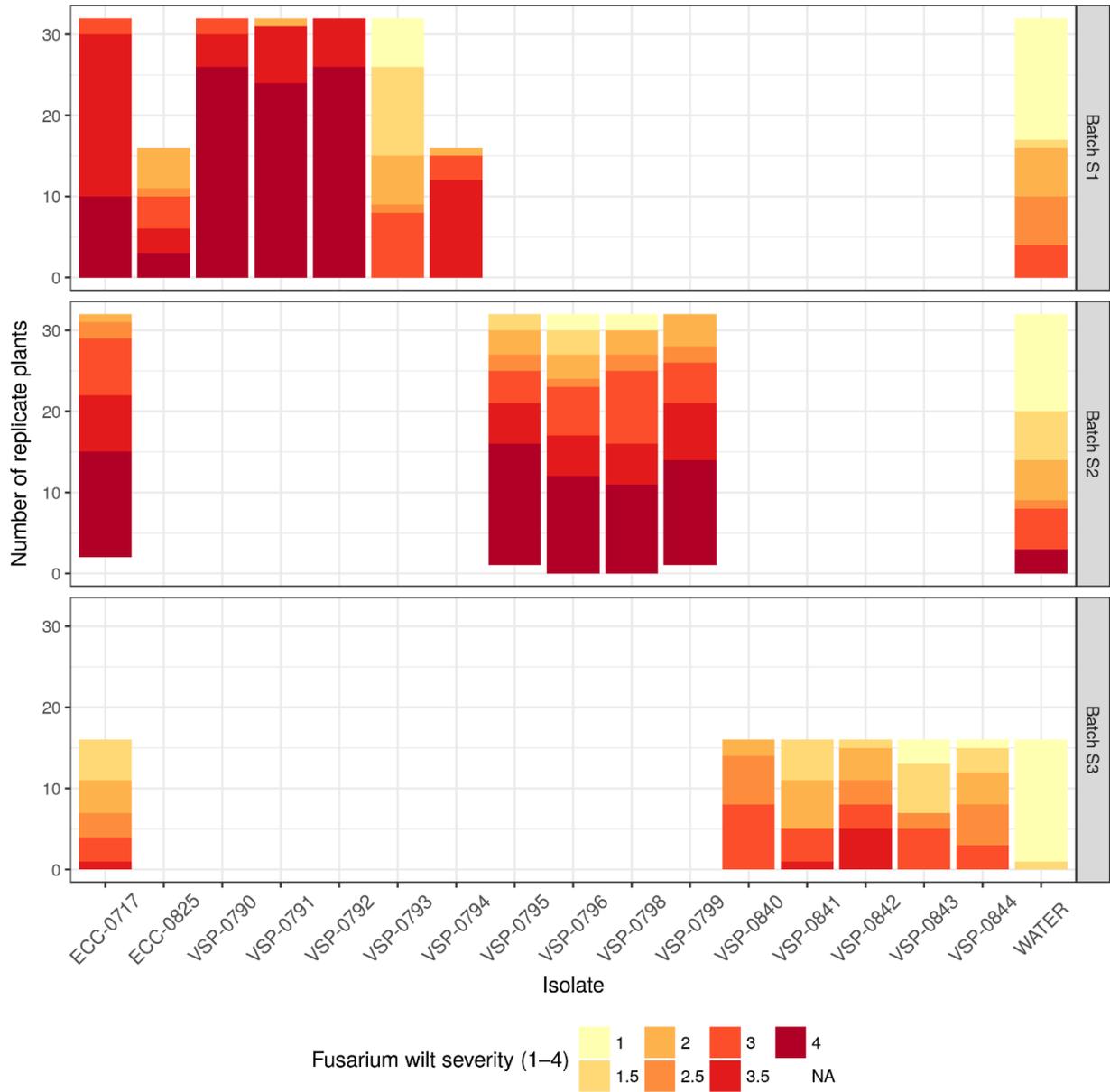
<sup>1</sup> Other = Butterhead, red leaf, or not available

<sup>2</sup> Diffs = a cultivar from the race differential set, Patriot (green leaf) or Banchu Red Fire (red leaf) planted in small demonstration plots as indicators

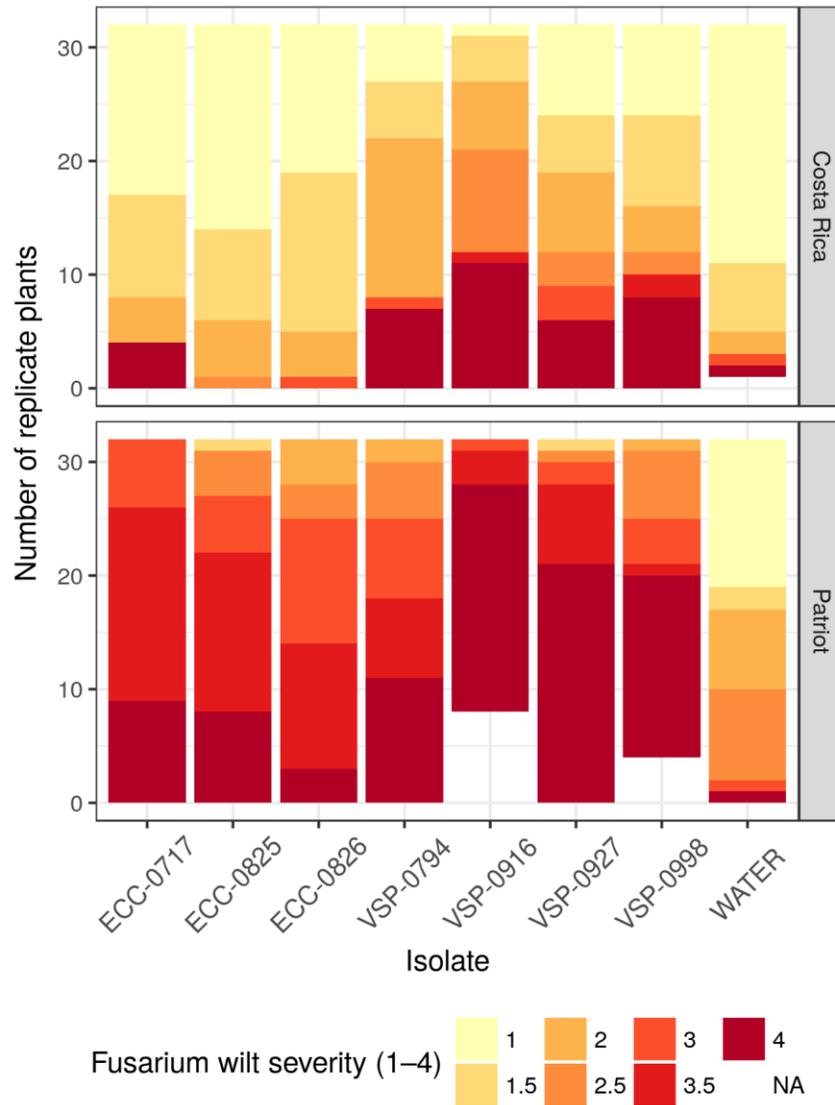
**Table 3.** Differential cultivar set for distinguishing races of *Fusarium oxysporum* f. sp. *lactucae*.

Cultivar	Race 1	Race 2	Race 3	Race 4
Costa Rica No. 4	R	S	S	S
Banchu Red Fire	S	R	S	R
Romana Romabella	R	R	S	S
Patriot	S	S	S	S

Minus (-) = no disease reaction (resistant); Plus (+) = disease occurs (susceptible)



**Figure 1.** Pathogenicity of *Fusarium oxysporum* isolates obtained in population survey on highly susceptible iceberg lettuce cultivar ‘Grizzly.’ Colored portions of each bar indicate the number of isolates assigned that disease severity rating. Data across two experimental repetitions was combined, but half-size bars indicate repetition has not been completed. Isolate ECC-0717 is a *Fusarium oxysporum* f. sp. *lactucae* race 1 reference strain used as a positive control, and plants inoculated with water served as a negative control.



**Figure 2.** Pathogenicity of *Fusarium oxysporum* isolates obtained in population survey on two of the *Fusarium* wilt race differential cultivars, Patriot and Costa Rica #4. Colored portions of each bar indicate the number of isolates assigned that disease severity rating. Data was combined across two experimental repetitions. Isolate ECC-0717 is a *Fusarium oxysporum* f. sp. *lactucae* race 1 reference strain used as a positive control, and plants inoculated with water served as a negative control.