Monitoring the population of the lettuce Fusarium wilt pathogen in California and occurrence of the disease with Pythium wilt

Final Report to the California Leafy Greens Research Program
April 1, 2022 to March 31, 2023

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Objectives

1. Collect FOL samples representing the population from outbreaks of concern in the Imperial Valley, Huron area, and 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

   Deliverable: race, vegetative compatibility group, and DNA sequence type of the collected FOL samples

3. Assess the frequency of concurrent Fusarium wilt and Pythium wilt symptoms in soilborne disease outbreaks in the Salinas Valley

   Deliverable: a list of fields with incidence of Fusarium wilt, Pythium wilt, both Fusarium and Pythium wilt, and other soilborne diseases with associated information (date of collection, anonymized location, lettuce type and cultivar)

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, but has recently increased in incidence and severity in coastal production regions. Little recent information is available on the FOL population in these regions, therefore an updated assessment is warranted. Furthermore, it is unclear if FOL is influencing the INSV-Pythium wilt epidemic. 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; 2) characterize the sample for pathogenic race, vegetative

compatibility group, and DNA sequence type; and 3) assess the frequency of concurrent Fusarium wilt and Pythium wilt symptoms in soilborne disease outbreaks in the Salinas Valley. For Objective 2, a total of 278 *F. oxysporum* isolates have been collected from 47 locations to date, with 68% and 21% of the isolates obtained from Monterey County and the Santa Maria Valley, respectively. A novel race variant was detected based on a resistant reaction to Banchu Red Fire that correlates with a susceptible reaction to the commercial cultivar San Miguel. A total of 49 isolates have been subjected to partial race typing to date using San Miguel as an unofficial indicator, of which 30 were identified to be the novel race variant. This variant was identified from 8 locations in the Salinas district and 2 locations in the Santa Maria district. All 11 isolates typed for vegetative compatibility group to date belong to the same group as race 1 isolates endemic to California and Arizona despite differences in race type. For Objective 3, a total of 23 location-cultivars (17 locations, several for which multiple cultivars were evaluated) were surveyed. At all locations, at least one plant exhibited visible symptoms of internal vascular discoloration (indicative of Fusarium or Verticillium wilts) or external root rot (indicative of Pythium wilt) separately. However, at only 12 locations were both types of symptoms observed on the same plant, and the number of plants exhibiting both symptoms ranged from 4% to 40% at these locations. In numerous cases, lab analysis of plants from surveyed locations could not confirm the disease that was suspected from the visual symptom assessment. Work to further confirm the novel race variant and evaluate isolates from more locations is ongoing. Analysis of Fusarium-Pythium coinfection surveys is ongoing but early results the need for a new study design for 2023.

Procedures, Results, and Discussion

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

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 278 *Fusarium oxysporum* isolates from 47 fields have been collected and stored in the lab (Table 2). A total of 62% and 20% of the isolates were obtained from iceberg and Romaine types, respectively (Table 2). A total of 68% and 21% of the isolates were obtained from Monterey County and the Santa Maria Valley, respectively.

<u>Objective 2 - Characterize race, phenotypic, and genetic variation of the sampled isolates</u>

In the course of the population survey (Objective 1), we received samples from locations in which reactions of cultivars to Fusarium wilt were inconsistent with FOL race 1. The criteria for suspecting this novel race variant are field observations of either a known susceptible cultivar exhibiting tolerance, a known tolerant cultivar exhibiting susceptibility, or both. Specifically, the iceberg cultivar San Miguel is known to be highly tolerant of FOL race 1, but has been observed in the field to be highly susceptible to the novel race variant. Preliminary race typing tests by both our lab and Dr. Richardson detected a differential reaction on Banchu Red Fire, one of the original four official differential cultivars (Table 1), but that Banchu Red Fire did not always provide a consistent reaction. Therefore, we used San Miguel in our race typing tests as an informal indicator. Furthermore, to improve throughput in our efforts to confirm the geographic distribution of the novel variant, we reduced the number of cultivars tested to two: San Miguel and the iceberg cultivar Grizzly as the susceptible control.

F. oxysporum isolates were subjected to pathogenicity testing against Grizzly and San Miguel using methods described by Paugh and Gordon (2020) and Scott et al. (2010). Briefly, lettuce seedlings at the 1-2 leaf stage were gently removed from the germination tray, the roots trimmed, and dipped in a suspension containing 1×10^7 spores mL^{-1} of FOL for 10 minutes. Inoculated seedlings were planted into 5" foam containers at three plants per pot with sterile potting mix and maintained in a greenhouse with a day/night cycle of 91ºF/73ºF. Pots were arranged in a randomized complete block with 4 replications. After 28 days, exterior symptoms of Fusarium wilt severity were assessed on a 1 to 4 scale where 1 = healthy and 4 = dead. Each isolate was evaluated in two repeated experiments. For this preliminary analysis, the criterion for pathogenicity on each cultivar was killing at least one of twelve replicate plants in both experimental repeats. Race type was assigned as follows: race 1 = pathogenic against Grizzly but not San Miguel; variant = pathogenic against both Grizzly and San Miguel; non-path = not pathogenic against either cultivar.

Race typing tests have been completed for 49 isolates, which originated from 12 locations in the Salinas district and 4 in the Santa Maria district. Of these, 30 isolates originating from 8 locations in Salinas and 2 in Santa Maria were determined to be the novel race variant (Table 3; Table 4). In all but one location, the greenhouse race typing results confirmed the suspicion from field observations of the novel race variant. The exception was location Salinas E in which both isolates were found to be non-pathogens against these two lettuce cultivars, however this location had a severe outbreak of INSV-Pythium. Additionally, at two locations we detected that both race 1 and the variant were present. Of the remaining isolates, 13 were determined to be race 1 and 6 isolates were not pathogenic against either cultivar. The locations tested to date were prioritized for screening based on anecdotal observations of commercial crops or demonstration trials. Therefore, the high percentage of variant isolates identified in the sample set to date likely represents a sampling bias. (Table 4).

A subset of isolates was also tested in preliminary experiments for pathogenicity against Banchu Red Fire. These experiments showed that Banchu Red Fire appears to be tolerant to the variant (Figure 1) and thus exhibits a differential reaction that is the inverse of San Miguel.

Reference isolates of FOL were obtained from Dr. Gordon's collection and from Dr. LeBlanc to be used as controls. Some of these isolates had previously been race typed against three (Patriot, Banchu Red Fire, and Costa Rica No. 4) of the four original differential cultivars (Paugh and Gordon, 2020). All tested isolates were determined to be race 1. In our experiments, however, we unexpectedly found that all 8 of the reference isolates tested to date caused mortality on San Miguel, which is consistent with the novel race variant (Table 5; Figure 2). Additional experiments are needed to evaluate the variant isolates, including the Gordon reference isolates, against the full differential cultivar set.

Although our experiment was designed to only detect qualitative differences in pathogenicity, non-statistical trends in virulence (quantitative disease severity) were observed. These trends suggest that the overall severity of disease caused by the variant in susceptible cultivar Grizzly was lower compared to race 1 isolates (Figure 2). This trend requires further confirmation in experiments designed for this purpose.

A subset of isolates was assigned to a vegetative compatibility group (VCG) using methods described by Correll et al. (1987) and Paugh and Gordon (2020). VCG is a method to characterize populations and in *F. oxysporum* often correlates with *forma specialis* and pathogenic race. Briefly, nitrogen non-utilizing (*nit*) mutants were obtained for each isolate by culturing on minimal media amended with chlorate. Each isolate was paired on minimal media with known tester strains, and the reaction between the isolates and testers was assessed after 14 days of growth. Reactions were scored on a 0 to 4 scale where 0 = no reaction, 1 = weakly positive reaction, and 4 = fully compatible reaction. Isolates were assigned to a VCG based on a minimum score of 1, and were assigned to a VCG subgroup based on a minimum score of 4. The experiment was conducted with two replicate mutants and at least two replicate plates. A total of 11 isolates have been subjected to VCG testing to date. Of these 11 isolates, all were assigned to *F. oxysporum* VCG 0300 (Table 3). To date, all FOL isolates from California and in a parallel project in Arizona have been assigned to VCG 0300.

<u>Objective 3 - Assess the frequency of concurrent Fusarium wilt and Pythium wilt symptoms in soilborne disease outbreaks in the Salinas Valley</u>

Fields were arbitrarily selected based on the presence of disease activity and a suspicion of Fusarium wilt. Plants with symptoms of a soilborne disease were arbitrarily selected for sampling. Plants were selected to represent mild to severe foliar disease symptoms based on a 0 to 4 scale where: 0 = healthy; 1 = slight outer-leaf chlorosis with stunting; 2 = more extensive chlorosis with stunting or a stunted head; 3 = severely stunting or no head formation; and 4 = dead. Most plants sampled were rated as 1, 2, or 3, and the number of plants sampled was approximately equal for these three ratings. Each plant was carefully uprooted by loosening the soil outside the root zone. Loose soil was removed from the root system by gently shaking the plant, and at approximately two-thirds of locations, more soil was removed by dipping the root system consecutively in two buckets with water. Symptoms of exterior root rot were assessed on a 0 to 4 scale where 0 = healthy without rot and 4 = 100% of exterior root area exhibits necrosis. Plants were cut longitudinally, and internal symptoms of vascular disease was assessed on a 0 to 4 scale where 0 = healthy and 4 = discoloration extending throughout the taproot or largest roots up to the leaves. Symptoms resembling INSV were assessed as present or absent from examination of the crown and midribs of sliced plants. Both the exterior of the root systems and sliced taproots were photographed for most sampled plants. A preliminary visual-based diagnosis was made based only on visible symptoms.

The plants used for the symptom survey were discarded, and three to five additional plants per location and cultivar, representing the symptoms seen in the survey, were sampled and brought to the lab. Samples of taproots with internal vascular symptoms were plated using standard plant pathology techniques onto Komada's semi-selective culture medium for isolation of *Fusarium*, and samples with symptoms resembling Verticillium wilt were plated onto Sorensen's NP-10 semi-selective culture medium for isolation of *Verticillium*. Secondary roots exhibiting root rot symptoms were plated onto PARP-V8 semi-selective culture medium for isolation of *P. uncinulatum*. Selected colonies were tentatively identified by microscopy, and the remainder of colonies were tentatively identified visually by culture morphology. Colonies of *F. oxysporum* were retained in long-term storage for molecular identification.

A total of 17 locations (i.e., plantings) were sampled in July to October 2022, and at several locations multiple cultivars were sampled resulting in 23 distinct locations and cultivars (Table 6). Of these, 16 location-cultivars were of iceberg and 7 were of Romaine. A total of 8 to 24 plants with symptoms of a soilborne disease were sampled per location. The visual symptom assessment was summarized to the proportion of plants with only internal vascular symptoms, with only external root rot symptoms, or with symptoms of both. At least one plant exhibited symptoms of Fusarium wilt or Pythium wilt for all location-cultivars. Plants with only internal vascular symptoms were observed at 17 location-cultivars, among which 11 also had plants with only root rot symptoms or both vascular and root rot symptoms and 6 location-cultivars had no root rot symptoms. At only two locations were no internal vascular symptoms observed. Root rot symptoms were strongly associated with presence of INSV symptoms, but internal vascular symptoms were not. For Romaine, the incidence of root rot symptoms was unexpectedly low even though INSV was present at all locations and they were surveyed between August 25 and September 21. Moreover, internal vascular symptoms were unexpectedly high for Romaine. However, the sample size of Romaine locations was low, and the 7 location-cultivars were distributed among only 3 physical ranches, raising the possibility that ranch- or cultivar-specific effects skewed our observations.

Although both vascular and root rot symptoms were visually observed at approximately two-thirds of location-cultivars (Table 6), this survey was designed to assess the distribution of symptoms among plants within fields and not among fields. At the field to field level, there is a strong sampling bias toward fields with suspected or known Fusarium wilt, and the INSV-Pythium wilt pressure throughout the Salinas Valley increased from the beginning (July 26) to end (October 21) of the survey period. Analysis of the data at the plants within field level is currently incomplete.

In a substantial number of cases, the pathogen that was suspected from visual symptoms could not be confirmed in the lab analysis. Of the 17 location-cultivars with vascular symptoms, FOL was confirmed at only 10 location-cultivars (Table 6). Whereas, a *Verticillium* species was isolated from 2 location-cultivars, and Verticillium wilt was suspected at an additional 2 location-cultivars for which the lab results are missing. At 15 location-cultivars with root rot symptoms, colonies resembling *Pythium uncinulatum* were observed from only 7 location-cultivars, whereas colonies resembling *Thielaviopsis basicola* (the pathogen that causes black root rot) were identified at the other 8 locations. *T. basicola* was also detected at 4 of the 7 *Pythium* locations. However, colonies resembling *Pythium uncinulatum* were observed at two location-cultivars at which no root rot symptoms were observed.

Taken together, the lab results suggest that visual assessment of symptoms may have limited utility and that future research should rely on lab culture-based methods for detection and identification. Isolates obtained from taproots and tentatively identified as FOL require further confirmation using molecular methods. Furthermore, a large number of colonies resembling *F. oxysporum* were obtained from secondary roots plated on to the *Pythium*-selective PARP-CMA medium (data not shown). Similar identification procedures are needed to determine the identity of these isolates.

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Table 1. Differential cultivar sets for distinguishing races of *Fusarium oxysporum* f. sp. *lactucae* and reactions of novel variant isolates observed to date.

Cultivar	Race 1	Race 2	Race 3	Race 4	Novel[1]
Original Differential Set[2]					
Patriot*	S	S	S	IR	S
Costa Rica No. 4*	HR	S	S	S	HR
Romana Romabella	HR	HR	S	IR	-
Banchu Red Fire	S	HR	S	IR	HR
New Differential Set[3]					
Gisela	S	S	S	S	-
Ballerina	S	S	S	IR	-
Lomeria	S	HR	HR	HR	-
Palmos	HR	S	IR	HR	-
Cultivars in this Study[4]					
Grizzly	S	-	-	-	S
San Miguel	HR	-	-	-	S

S = susceptible; IR = intermediate resistance; HR = highly resistant; - = not tested in this research

[1] Reactions of the novel race variant isolates on differential cultivars observed to date

[2] Reactions for original differential set were updated by International Seed Federation (ISF) in May 2022

[3] In the most recent ISF update (Nov. 2022), these four cultivars were combined with two cultivars from the original set to form a new set of six cultivars

[4] Reactions based on field observations of FOL endemic to coastal California

* Retained in the new differential set (Nov. 2022)

Table 2. Summary of *Fusarium oxysporum* isolates collected in project years 1 and 2.

		Standard[1]					Possible Variant[1]					Isolates
			Isolates					Isolates				
District[2]	**Sub-Region**	**# Fields**	**Ice-berg**	**Rom-aine**	**Oth-er[3]**	**Sub-Total**	**# Fields**	**Ice-berg**	**Rom-aine**	**Oth-er[3]**	**Sub-Total**	**Total**
Central Val.	San Joaquin	2	15			15						**15**
Low Desert	Imperial Valley	2	15			15						**15**
Salinas	Mid County	8	15	27	10	52	1	3			3	**55**
Salinas	North County	15	35	19	18	72	7	37	2	11	50	**122**
Salinas	South County	1		3		3						**3**
Salinas	n/a	3	3		4	7	1	3			3	**10**
Santa Maria	Lompoc Valley	1	9			9						**9**
Santa Maria	Santa Maria Val.	2	2		3	5	3	30		8	38	**43**
Santa Maria	n/a	1	6			6						**6**
	Total	**35**	**100**	**49**	**35**	**184**	**12**	**73**	**2**	**19**	**94**	**278**

[1] Classification based on observations of cultivar performance at that location. **Standard** = obtained from fields for which cultivar performance has not been tested, or cultivar performance has been tested and matches expectations. **Possible variant** = obtained from fields where certain cultivars exhibited an unexpected reaction (e.g., known tolerant cultivar appears susceptible, and/or known susceptible cultivar appears tolerant) either as a commercial field variety or in unreplicated demonstration trials

[2] Districts include adjacent valleys: Salinas includes the Pajaro Valley and San Benito County

[3] Other = Butterhead, red leaf, not available, or 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. Race type, vegetative compatibility, and sample origin information.

Isolate	Variety	Date Collected	District/Site Code	Race type[1]	VCG[2]
787	n/a	Sep 2021	Salinas	race 1	
788	n/a	Sep 2021	Salinas	race 1	
789	n/a	Sep 2021	Salinas	race 1	
966	Primo	Jul 2022	Salinas	race 1	
971	San Miguel	Aug 2022	Salinas	variant	
972	San Miguel	Aug 2022	Salinas	variant	
973	San Miguel	Aug 2022	Salinas	variant	
793	Tiber II	Sep 2021	Salinas A	non-path	
794	Tiber II	Sep 2021	Salinas A	variant	0300
826[3]	Tiber II	n/a	Salinas A	variant	
1042	Banchu Red Fire	Aug 2022	Salinas A	variant	
1057	Primo	Aug 2022	Salinas A	variant	
1058	Primo	Aug 2022	Salinas A	variant	0300
925	Paraiso	Jun 2022	Salinas A-2	variant	
926	Paraiso	Jun 2022	Salinas A-2	variant	
927	Paraiso	Jun 2022	Salinas A-2	variant	
825[3]	Hercules	n/a	Salinas B	variant	
791	San Miguel	Sep 2021	Salinas C	race 1	
795	Hercules	Sep 2021	Salinas C	variant	0300
796	Hercules	Sep 2021	Salinas C	variant	0300
797	Hercules	Sep 2021	Salinas C	variant	
799	Hercules	Sep 2021	Salinas C	variant	
929	Outfitter	Jun 2022	Salinas E	non-path	
930	Outfitter	Jun 2022	Salinas E	non-path	
932	Hercules	Jun 2022	Salinas F	variant	
933	Hercules	Jun 2022	Salinas F	variant	
934	Hercules	Jun 2022	Salinas F	variant	
935	San Miguel	Jun 2022	Salinas F	variant	
938	Primo	Jun 2022	Salinas F	variant	
959	San Miguel	Jul 2022	Salinas G	variant	
960	San Miguel	Jul 2022	Salinas G	non-path	
961	San Miguel	Jul 2022	Salinas G	non-path	
962	San Miguel	Jul 2022	Salinas G	variant	

Isolate	Variety	Date Collected	District/Site Code	Race type[1]	VCG[2]
1120	Hercules	Oct 2022	Salinas I	variant	
1128	Hercules	Oct 2022	Salinas I	non-path	
995	Telluride	Aug 2022	Santa Maria	race 1	
840	n/a	Oct 2021	Santa Maria A	variant	
841	n/a	Oct 2021	Santa Maria A	variant	
998	San Miguel	Jul 2022	Santa Maria A	race 1	
1082	Patriot	Sep 2022	Santa Maria A	variant	
1087	San Miguel	Sep 2022	Santa Maria A	variant	
1092	Fredonia	Sep 2022	Santa Maria A	race 1	
1093	Primo	Sep 2022	Santa Maria A	race 1	
916	Telluride	May 2022	Santa Maria B	race 1	0300
918	Telluride	May 2022	Santa Maria B	race 1	
919	Telluride	May 2022	Santa Maria B	race 1	
920	Telluride	May 2022	Santa Maria B	race 1	
974	Journey	Aug 2022	Santa Maria C	variant	0300
975	Journey	Aug 2022	Santa Maria C	variant	0300
Incomplete					
965	Banchu Red Fire	Jul 2022	Salinas	n/a	0300
996	Telluride	Aug 2022	Santa Maria	n/a	
1056	Paraiso	Aug 2022	Salinas A	n/a	
792	San Miguel	Sep 2021	Salinas C	n/a	0300
798	Hercules	Sep 2021	Salinas C	n/a	
790	confidential	Sep 2021	Salinas D	n/a	
931	Outfitter	Jun 2022	Salinas E	n/a	
997	Paraiso	Jul 2022	Santa Maria A	n/a	
917	Telluride	May 2022	Santa Maria B	n/a	

[1] Race type was determined according to the following: race 1 = pathogenic against Grizzly but not San Miguel; variant = pathogenic against both Grizzly and San Miguel; non-path = not pathogenic against either cultivar

[2] Assigning isolates to vegetative compatibility group (VCG) is a method to characterize fungal populations and in *F. oxysporum* often correlates with forma specialis and pathogenic race

[3] Isolate obtained from Kelley Richardson and Nick LeBlanc

Table 4. Pathogenicity of *Fusarium oxysporum* on lettuce summarized by location.

District[1]	Location[2]	Number of Isolates						
		Status				Race type[3]		
		Total	No data	Dis-carded	To Redo	Non-path	Race 1	Variant
Salinas	1	3	0	0	0	0	3	0
Salinas	2	7	5	0	1	0	1	0
Salinas	A	22	15	0	1	1	0	5
Salinas	A-2	8	5	0	0	0	0	3
Salinas	B	1	0	0	0	0	0	1
Salinas	C	9	2	0	2	0	1	4
Salinas	D	1	0	0	1	0	0	0
Salinas	E	3	0	0	1	2	0	0
Salinas	F	7	2	0	0	0	0	5
Salinas	G	4	0	0	0	2	0	2
Salinas	H	3	0	0	0	0	0	3
Salinas	I	14	12	0	0	1	0	1
Santa Maria	3	2	0	0	1	0	1	0
Santa Maria	A	28	20	0	1	0	3	4
Santa Maria	B	5	0	1	0	0	4	0
Santa Maria	C	6	4	0	0	0	0	2
TOTAL	**16**	**123**	**65**	**1**	**89**	**6**	**13**	**30**

[1] Districts include adjacent valleys: Salinas includes the Pajaro Valley and San Benito County; Santa Maria includes Lompoc and Oceano.

[2] Each location code refers to a specific ranch and lot. Locations without pathogenicity data are not included.

[3] Pathogenicity of *F. oxysporum* was assessed against the iceberg cultivars Grizzly (believed to be susceptible to all races including race 1 and the variant) and San Miguel (highly tolerant to race 1 and susceptible to the variant). Race type was determined according to the following: race 1 = pathogenic against Grizzly but not San Miguel; variant = pathogenic against both Grizzly and San Miguel; non-path = not pathogenic against either cultivar.

Table 5. Race type, vegetative compatibility, and sample origin information of reference isolates.

Isolate	Other Name	Date Collected	Location	Race type[1]	VCG[2]
717	JCP-043	1990	Huron	variant	
718	JCP-073	2002	Huron	variant	0300
719	JCP-024	2004	Wellton	variant	
720	GL-1815	2016	Chualar	variant	
721	GL-1515	2015	Davis	n/a	
722	GL-1639	2015	Watsonville	variant	0300
723	JCP-164	2002	Huron	variant	
827	NRRL 26844	1990	Huron	variant	
828	NRRL 26845	1990	Huron	variant	

[1] Race type was determined according to the following: race 1 = pathogenic against Grizzly but not San Miguel; variant = pathogenic against both Grizzly and San Miguel; non-path = not pathogenic against either cultivar

[2] Assigning isolates to vegetative compatibility group (VCG) is a method to characterize fungal populations and in *F. oxysporum* often correlates with *forma specialis* and pathogenic race

Table 6. Summary of survey of lettuce fields in the Salinas Valley in 2022 based on visual symptoms and lab culturing.

					Field - % Plants with Symptoms (+ or – INSV)[1]						Lab - % Positive Cultures[2]				
					Fusarium		Pythium		Fus+Pyth						
Type	**Date**	**Loc**	**Cultivar**	**Plants**	**+**	**-**	**+**	**-**	**+**	**-**	**Plants**	**Fus-arium**	**Pyth-ium**	**Black RR**	**Vertic illium**
Iceberg	Jul 26	A	Spyglass	24		96				4	3	100			
Iceberg	Jul 27	B	Newcastle	10		80					3	100			
Iceberg	Jul 27	B	Salute	10		100					3	100			
Iceberg	Jul 27	C	n/a	12		8	33		17		0				
Iceberg	Aug 9	D	Blackhorse	21	5						4	100			
Iceberg	Aug 10	E	San Miguel	21			67		5		5	100	0	20	
Iceberg	Aug 26	G	Armstrong	12			42				3	0	0	33	
Iceberg	Aug 26	G	Chalone	11	9	9	9	9	18		3	0	0	67	67
Iceberg	Aug 26	G	San Miguel	7	14		43				3	0	33	33	
Iceberg	Aug 26	G	Telluride	7			14		29		3	0	0	100	100
Iceberg	Sep 8	I	Salute	8	12	38	12		25		3	0	0	33	?
Iceberg	Sep 8	J	Newcastle	8	38	12	38				2	0	0	50	?
Iceberg	Oct 6	N	n/a	16	6	12			25	12	9	83	25	0	
Iceberg	Oct 7	O	San Miguel	12			8				6	0	67	0	?
Iceberg	Oct 7	P	Hercules	10	20	20			40		8	62	12	12	
Iceberg	Oct 21	Q	Salute	12			33	17		17	7	29	57		
Romaine	Aug 25	F	n/a	24	4	12	25		4		6	0	50	33	0
Romaine	Sep 7	H	Solid King	12	25	50	8		17		4	100	25	0	
Romaine	Sep 8	K	n/a	10			80	10	10		2		0	100	
Romaine	Sep 21	L	Honolua	10	30	10					5	0	40	40	
Romaine	Sep 21	L	True Heart	9	22	22	22				4	0	0	67	
Romaine	Sep 21	M	Arroyo	9	11	22					7	71	67	0	
Romaine	Sep 21	M	Momentus	10	10	50					8	75	0	0	
TOTAL/AVERAGE		17	23 (Loc-Cult)	285	16	36	31	12	19	11					

[1] Visual diagnosis of Fusarium wilt was estimated by slicing open the taproot and examining interior symptoms, and visual diagnosis of Pythium wilt was estimated from assessments external root surfaces. Diagnoses: INSV presence/absence was assessed from visual symptoms only.

[2] Laboratory diagnosis was determined by macroscopic morphology in semi-selective culture media with comparisons to known cultures.

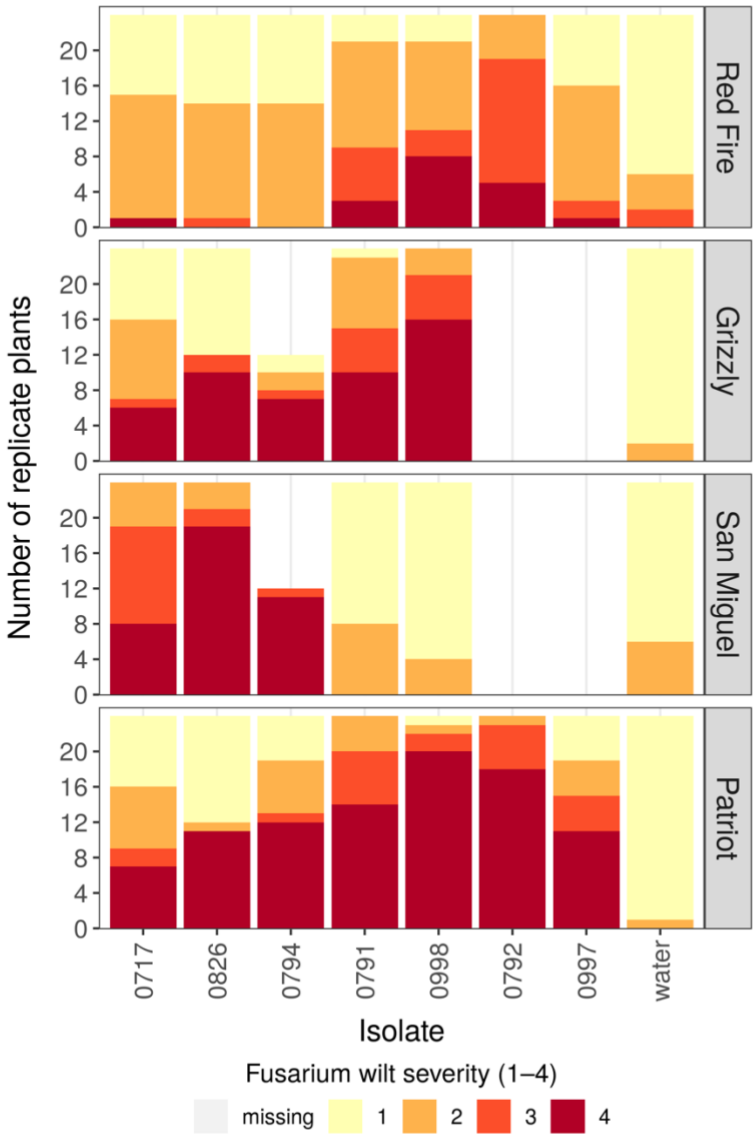

Figure 1. Race typing of *F. oxysporum* in greenhouse experiments. Patriot (susceptible to all races) and Banchu Red Fire (susceptible to race 1) are included in the official differential cultivar set, and Grizzly (believed to be susceptible to all races) and San Miguel (highly tolerant to race 1) are used as unofficial indicators. Foliar symptoms of Fusarium wilt severity were assessed on a 1 to 4 scale where 1 = healthy and 4 = dead. Colored segments of bars indicate the number of plants with the given Fusarium wilt severity rating. Data are from separate experiments: the combined data from two repeats of the same experiment with Grizzly and San Miguel, and a single repeat of an experiment with Patriot and Banchu Red Fire. Plants were dipped in a spore suspension and transplanted into four replicate pots each with three plants.

Figure 2. Pathogenicity of *F. oxysporum* in greenhouse experiments to the lettuce cultivars Grizzly (believed to be susceptible to all races) and San Miguel (highly tolerant to race 1) used as unofficial indicators. Plants were dipped in a spore suspension and transplanted into four replicate pots each with three plants. Foliar symptoms of Fusarium wilt severity were assessed on a 1 to 4 scale where 1 = healthy and 4 = dead. Colored segments of bars indicate the number of plants with the given Fusarium wilt severity rating. Batches of isolates were evaluated in separate experiments each consisting of six unknown isolates, a positive control, and a negative water control, but are presented here in a combined summary. Environmental conditions in the greenhouse varied across batches, therefore data are presented separately based on time of year to reflect differences in disease pressure. Data across two experimental repetitions was combined, but half-size bars indicate repetition has not been completed.