Layman's summary

CALIFORNIA CELERY RESEARCH ADVISORY BOARD
RESEARCH PROJECT REPORT

for the period of:
October 1, 2013 - September 30, 2014

PROJECT TITLE: Plant Pathology Support for the Celery Breeding Program (on resistance to Fusarium Yellows, caused by *Fusarium oxysporum* f. sp. *apii*)

PROJECT LEADER: Lynn Epstein, Department of Plant Pathology, University of California, Davis, California 95616

COOPERATING PERSONNEL: Sukhwinder Kaur, Vincent D'Antonio, Peter Chang, Noelia Carrasquilla-Garcia and Doug Cook

Fusarium oxysporum f. sp. *apii* (*Foa*) race 1 was first reported on celery with Fusarium yellows in 1914; it is virulent on self blanching celery cv. Golden but avirulent on green cultivars such as Tall Utah. In 1976, *Foa* race 2 was identified; it is virulent on cv. Tall Utah, but asymptomatic to weakly virulent on cultivars (e.g. cv. Challenger) with the celeriac-derived gene *Fu1*. A race 3 that was virulent on Tall Utah but avirulent on both cv. Golden and on cv. Challenger also was reported. Between 1993 and 2014, we isolated *F. oxysporum* from celery crown and root tissue that had symptoms of Fusarium yellows, primarily in California. DNA sequence (628 bp from an intron-rich EF-1α and 640 bp from rDNA IGS) were determined for 239 isolates. Between 1993 and 2012, all isolates that caused the classic orange-brown discoloration were a clonal and invariant population of *Foa* race 2; this sequence has not been reported previously. In addition to *Foa* race 2, two other *F. oxysporum* lineages also were isolated from symptomatic tissue; these isolates do not appear to cause symptoms and while related to each other, are variable in sequence. In 2013, a new race 4 was discovered in three celery production fields in Camarillo, California; race 4 isolates are highly virulent on both Tall Utah and the previously resistant Challenger. Two additional celery fields in Camarillo with *Foa* race 4 were identified in 2014. Based on the the EF-1α and rDNA IGS amplicons, race 4 is related to race 3, which is in "clade 1,"as are all of the previously described *Foa*. Next generation sequencing was done with Ilumina Mi-Seq paired-end reads to 10X coverage on 15 of the isolates (six race 2 isolates selected over time and location), one race 4 isolate, six of the polymorphic non-pathogenic clade 5 isolates, and two of the non-pathogenic clade 1 isolates. Sequences were both *de novo* assembled and assembled against a full genome-sequenced *F. oxysporum* strain 4287, which infects tomato and is known as race 2. We are primarily using the next generation sequence to generate PCR primers that we can use to Analysis of single nucleotide polymorphisms with *structure* software indicate that support the contention that race 2 has been relatively invariant in California between 1993 and 2014, that race 4 is relatively distantly related to race 2, and that the variable non-pathogenic isolates are in a distinct clade.

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II. OBJECTIVES**:**
The purpose of the research in this proposal is to improve and expedite the selection and breeding effort in order to provide long-term control of Fusarium yellows and other critical fungal diseases in California celery.

Specific objectives:

1. To determine if the unusually severe disease 2013 CCRAB field trial in Oxnard was due to either a change in the predominant isolate, unusually high inoculum in the soil, and/or, to unusual environmental conditions.
2. To complete the next generation sequencing of 15 isolates of *Fusarium oxysporum* from celery grown in California with Fusarium yellows
3. To develop a DNA-based assay for determination of *F. oxysporum f. sp. apii* (*Foa*) inoculum concentration in soil that will be useful to growers
4. To continue to optimize a greenhouse assay for resistance to *Foa* so that 1) we can have multiple cycles of screening per year, 2) we can increase the inoculum concentration of *Foa* and consequently can screen under more intense disease pressure than has typically occurred in field trials in most years, and 3) we have a quantitative measure of resistance to *Foa* so that we can more sensitively detect effects of different resistance genes and additive effects of quantitative resistance genes.
5. To determine if we can differentiate between resistance to *Foa* from the major dominant gene derived from celeriac and other sources of Fusarium-resistance.

RESULTS

Objective 1(A). Identification of *Fusarium oxysporum* f. sp. *apii* race 4 as the causal agent at the 2013 CCRAB trial.

The severity of *Foa* is to some extent always dependent on environmental conditions; we presume that insect injury to the crown and roots, and water stress can exacerbate the disease. Nonetheless, a new race of *Foa*, which we call race 4, has emerged in the Camarillo CA area. We detected race 4 in three fields in Camarillo in 2013 and in two additional fields in 2014 (Table 1). In the field, the pathogen seems extremely aggressive and appears to kill younger plants than the historic race 2. Once infected, race 4 appears to more rapidly degrade the crown

into a more watery rot than does race 2. Races are characterized by their virulence on differential hosts. Race 4 is highly virulent on Challenger, other UCD breeding material, and as far as we know, current commercial lines (Table 2).

Table. 1.

Fusarium oxysporum f. sp. *apii* races reported in California

Race	Virulent on Golden Self-Blanching	Virulent on Tall Utah[a]	Virulent on Challenger[b]	First "report"	Relative importance: 1990 – 2014 in CA
1	+	-	-	?[c]	Unknown in CA
2	+	+	-	1976[d]	Dominant
3	-	+	-	1984	Very minor
4	+	+	+	2013	Only in Camarillo, CA
Non-pathogenic	-	-	-		None? Apparently non-pathogenic

[a]Tall Utah was introduced in late 1950's.
[b]Resistance from celeriac was first introduced in ca. 1984 by Orton et al.
[c]*F. oxysporum* f. sp. *apii* was first reported in 1914.
[d]Orro et al. 1976. A crown rot of celery. Calif. Agric. 30(6): 10-11.

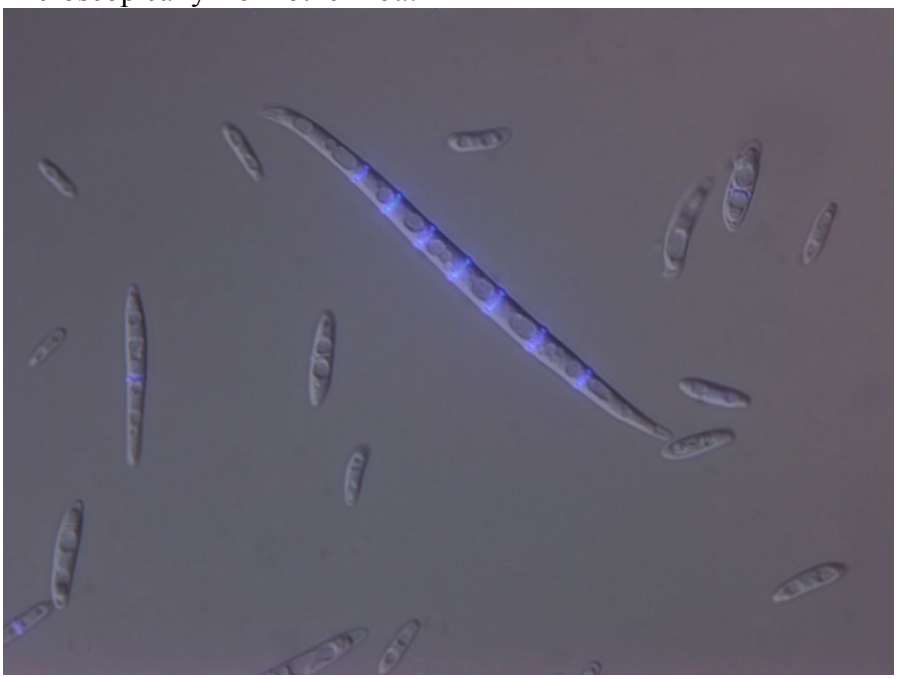
Table. 2.

Disease ratings of the four *Foa* races on differential cultivars

Race	Isolate	Disease rating, 0 none to 5 dead[a]		
		Cultivar		
		Golden Self Blanching	Tall Utah 52-70 Improved	Challenger
(Mock)	-	0.3	0.1	0.1
1	250-7 (Michigan)	1.4*	0.1	0.3
2	247-1A	3.0****	2.0****	0.1
3	NRRL 38295	0.9	1.7****	0.3
4	274-4C	2.8****	3.0****	3.3****
(Non-pathogenic Clade 1)	295	0.9	0.1	0.2
(Non-pathogenic Clade 5)	273-2M2	0.6	0.4	0.3

[a]Within each column, values were not significantly different from the Mock control by Dunnet's test. *, P=0.03; **** P<0.0001.

Fig. 1. A microscopic image of *Fusarium oxysporum* f. sp. *apii* race 4 with a macroconidium (in center) surrounded by (mostly) microconidia. Conidia were stained with the fluorescent dye uvitex, which stains the cell walls in the macroconidium blue. *Foa* race 4 is indistinguishable microscopically from other *Foa.*



Race 4 can be readily distinguished from other strains of *Fusarium oxysporum* by DNA sequence. Figs. 2 and 3 show the DNA sequence of two amplicons that are often used to differentiate *Fusarium oxysporum* isolates: a 628 bp from an intron-rich EF-1α and a 640 bp from rDNA IGS. While we note that this 1268 bp is not sufficient to distinguish either race 4 or race 2 from every known isolate of *Fusarium oxysporum* (http://isolate.fusariumdb.org), the sequence clearly distinguishes what appear to be the key strains in the epidemiology of Fusarium yellows on celery in California.

Because there were large quantities of yellow nutsedge in all the fields with *Foa* race 4, growers' postulated that nutsedge was an alternate host for *Foa* race 4. However, although there were lesions on the nutsedge roots in a field with celery and *Foa* race 4, we did not isolate any apparent F. oxysporum from the nutsedge. The source of the *Foa* race 4 is unknown. We do know that race 4 shares an identical 1268 sequence ef1-alpha and rDNA IGS with *Foa* race 3, which was present in California in the 1980's (Table 1). Based on our isolation results between the 1990's and the present, race 3 is not a major component in *Foa*-celery interactions—we have never isolated it, and our only race 3 isolate was obtained from a culture collection.

Fig. 2. The DNA sequence of concatenated ef1-alpha and rDNA IGS amplicons of *F. oxysporum* f. sp. *apii* race 4.

>Foa_274-4C_race4_CA_celery

GTCGACTCTGGCAAGTCGACCACTGTGAGTACTCTCCTCGACAATGAGCATATCTGCCATCGTCAATCCCGACCAAG
ACCTGGCGGGGTATTTCTCAAAGTCAACATACTGACATCGTTTCACAGACCGGTCACTTGATCTACCAGTGCGGTGG
TATCGACAAGCGAACCATCGAGAAGTTCGAGAAGGTTAGTCACTTTCCCTTCAATCGCGCGTCCTTTGCCCATCGAT
TTCCCCTACGACTCGAAACGTGCCCGCTACCCCGCTCGAGACCAAAAATTTTGCAATATGACCGTAATTTTTTTGGT

GGGGCACTTACCCCGCCACTTGAGCGACGGGAGCGTTTGCCCTCTTACCATTCTCAGAACCTCAATGAGTGCGTCGC
ACGTGTCAAGCAGTCACTAACCATTCAACAATAGGAAGCCGCTGAGCTCGGTAAGGGTTCCTTCAAGTACGCCTGGG
TTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTATCACCATCGATATTGCTCTCTGGAAGTTCGAGACTCCTCGC
TACTATGTCACCGTCATTGGTATGTTGTCGCTCATGCTTCATTCTACTTCTCTTCGTACTAACATATCACTCAGACG
CTCCCGGTCACCGTTGTTACGATCTGCTGAGGGTAAGCCGTCCTTCGCCTCGATTTCCCCAATGGGTTCTCCGGATT
TCTGGAGACTTGTAGGGGTTGTGGGATTTTTGATGTGTCGTCTCCGGACGGGCGGTGCAGGGTAGTCGAGTTAGACT
TGGTGGAATTCCGTCGATAGGAGTTCCGTCGAGTCTGGTCGGCTGTGTGTTGGACGGTGCAGGGTAGGCTGCTTGGA
CATGGTCGGGTCGAGGGTCGATTCGAGGGCCGGCCTGTCGATGATGTGTGATGTATGCGGTCTAGGGTAGGCTGGTT
TGTCTTGGTCCAATTTGATGTCGGCTCCCGTGCAGGCCAGAGTGAGGGGGGTCCAGGGTAGGTACAGGGTAGGCAGC
TTAGATTTGGCCGATCTGGAGGTCGATTCTCCGGCCGGCGGATCTGACACTGTCAAAACGAGGTGCGAGCGGTGTAG
GGTAGGCTAGTTTCGTCCTCGCCAGGTTGCGATTTGGACGAGATATGTGGTCTAGGGTAGGCTCTAGGGTAAGTAGA
ATTCGAGTTTCGTCGCCGACAGTTTTCTGTGGGTGTGTGGTAGGTACAGGGTAGGCAAATCTCCCTCCGGCCAGTAC
TTGTCTGGTTGGTCGTGAGTCGATTTTTTTGTTTT

Fig. 3. The DNA sequence of concatenated ef1-alpha and rDNA IGS amplicons of *F. oxysporum* f. sp. *apii* race 2.

>Fo_258-1B_race2_CA_celery
GTCGACTCTGGCAGTCGACCACTGTGAGTACTCTCCTCGACAATGAGCTTATCTGCCATCGTCAATCCCGACCAAGA
CCTGGTGGGGTATTTCTCAAAGTCAACATACTGACATCGTTTCACAGACCGGTCACTTGATCTACCAGTGCGGTGGT
ATCGACAAGCGAACCATCGAGAAGTTCGAGAAGGTTAGTCACTTTCCCTTCGATCGCGCGTCCTTTGCCCATCGATT
TCCCCTACGACTCGAAATGTGCCCGCTACCCCGCTCGAGACCAAAAATTTTGCAATATGACCGTAATTTTTTTTGGT
GGGGCACTTACCCCGCCACTTGAGCGACGGGAGCGTTTGCCCTCTTAACCATTCTCACAACCTCAATGAGTGCGTC
GTCACGTGTCAAGCAGTCACTAACCATTCAACAATAGGAAGCCGCTGAGCTCGGTAAGGGTTCCTTCAAGTATGCCT
GGGTTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTATCACCATCGATATTGCTCTCTGGAAGTTCGAGACTCCT
CGCTACTATGTCACCGTCATTGGTATGTTGTCGCTCATGCTTCATTCTACTTCTCTTCGTACTAACATATCACTCAG
ACGCTCCCGGTCACCGTTGTTACGATCTGCTGAGGGTAAGCCGTCCTTCGCCTCGATTTCCCCAATGGGTTCTCCGG
ATTTCTGGAGACTTGTAGGGGTTGTGGGATTTTTGATGTGTCGTCTCTGGACGGGCGGTGCAGGGTAGTCGAGTTAG
ACTTGGTGGAATTCCGTCGATAGGAGTTCCGTCGAGTCTGGTCGGCTGTGTGTTGGACGGTGCAGGGTAGGCTGCTT
GGACATGGTCGGTTCAAGGATCGATTCGAGGGCCGGCGCCTGTCGATGATGTGTGATGTATGCGGTCTAGGGTAGGC
TGGTTTGTCTTGGTTCAATTTGATGTCGGCTCCCGTGCAGGCCAGAGTGAGGGGGGTCCAGGGTAGGTACAGGGTAG
GCAGCTTATATTTGGTCGATCTGGAGGTCGATTCTCCGGCTGGCGGATCTGACACTGTCGAAACGAGATGCGAGCGG
TGTAGGGTAGGCTAGTTTCGTCCTCGCCAGGTTGCGATTTGGACGAGATATGTGGTTTAGGGTAGGCTCTAGGGTAA
GTAGAATTCGAGTTTCGTCGCCGACAGTTTTCTGTGGGTGTATGGTAGGTACAGGGTAGGCAAATCTCTCTCCGGCC
AGTACTTGTCTGGTGGTCGTGAGTCGATTTTTTTGTTTT

Objective 1B. **Screening of the UCD germplasm collection for resistance to race 4.** The 2013 CCRAB trial in race 4 soil resulted in 100% loss of all commercial cultivars and all of the current UCD breeding lines. Given that we had a new pathogen with no known resistance to Foa race 4, we added an objective to immediately start screening in the greenhouse for germplasm resistant to *Foa* race 4. Table 1 shows results as of 25 Nov. 2014. Cultivars that were screened in two trials, some of which were designed to examine reproducibility of results, are shown with matching shading. Some UCD germplasm material was included as a reference rather than as potential germplasm. Accessions in bold currently are being tested in the field against race 4 by Larry Pierce (Table 4). Conclusions are as follows. 1) Although we are continuing to try to reduce trial-to-trial variability, our results are at least as reproducible as those historically obtained from field trials. 2) Finding resistance against race 4 will be a much greater challenge than finding resistance to race 2 (see a much greater percentage of acceptable ratings in Table 6 than in Table 3). 3) Nonetheless, we have five accessions that are currently being field tested as indicated in Table 4. Results from the first field trial may help us to prioritize our testing of other accessions in the UCD germplasm collection; 356 accessions are listed. We are attempting to increase the number of cultivars tested in 2014.

Table 3. Performance of selected accessions in the greenhouse assay against *F. oxysporum* f. sp. *apii* race 4. Ten plants were assayed per trial for both the race 2-inoculated and mock-inoculated.

Cultivar/ Accession	Crop	Trial	Avg. vascular discoloration rating, 0 (none) to 5	Fraction of height of mock-inoculated controls, excluding dead plants	Fraction of plants that died	**Mock-inoculated controls**: avg. vascular discoloration rating, 0 (none) to 5	Avg aerial height of plant, cm
A0165	Celeriac	8	0	1.36	0	0	12.2
A0165	Celeriac	3	0.4	0.75	0	0.3	21.2
A0235	Unknown	3	0	1.07	0	0	39.2
A0235	Unknown	8	0.4	1.19	0	0	15.7
A0163	Celery	8	0.2	1.39	0	0	22.7
A0103	Smallage	8	0.3	1.02	0	0	22.7
A0021	Celeriac	8	0.4	0.95	0	0	17.7
A0102	Celeriac	8	0.4	1.14	0	0	17.8
A0102	Celeriac	3	0.5	1.06	0	0	29.8
A0136	Celeriac	2	0.6	0.65	0	0	33.3
A0136	Celeriac	6	3.3	0.97	0.2	0	27.6
A0105	Celeriac	8	1	1.26	0	0	20.0
A0210	Celeriac	2	1.2	0.90	0	0	30.3
A0210	Celeriac	6	3.9	0.61	0.3	0	18.5
A0112	Celeriac	3	1.5	1.02	0	0	30.9
A0112	Celeriac	2	2.4	0.91	0	0	34.4
A0140	Celeriac	3	1.5	1.10	0	0	24.0
A0140	Celeriac	2	2.3	0.71	0.1	0	26.1
A0172	*A.g.*var.*lusitanicum* -Wild	8	1.6	0.85	0.1	0	15.3
A0127	Celeriac	2	1.9	1.00	0	0.3	33.9
Sabroso	Celery	2	1.9	0.92	0.1	0.2	29.8
Sabroso	Celery	3	3.2	.	0.1	0	24.9
A0027	Celeriac	2	2.1	.	0.1	0	31.3
UC040A	Celery	2	2.1	0.69	0.2	0	22.3
A0162	*A.leptophyllum*- Wild	8	2.2	0.90	0.2	4	15.3
A0146	Celeriac	2	2.4	0.73	0	0	28.8
A0169	Celeriac	3	2.5	1.17	0	0	22.9
UC136VF	Celery	6	2.5	0.37	0.3	0	25.5
A0009	Celery	6	2.6	0.44	0.2	0	25.9
A0137	Celeriac	6	2.7	1.12	0.3	0.8	22.3
A0020	Smallage	8	2.9	1.13	0	0	19.2
A0328	Celeriac	8	2.9	.	0.1	0	16.0
UC390S-2	Celery	2	2.9	0.58	0.1	0	24.9
UC390S-2	Celery	3	2.9	0.91	0	0	23.8

A0173	Celeriac	3	3.1	0.38	0	0.1	17.1
A0321	Celery	8	3.2	1.00	0	0	16.6
Challenger	Celery	3	3.2	0.81	0	0	27.7
Challenger	Celery	2	3.4	.	0.1	0.2	27.6
Challenger	Celery	6	4.6	0.63	0.8	0	22.5
Tall Utah 52-70R improved	Celery	2	3.2	.	0.3	0	20.7
Tall Utah 52-70R improved	Celery	3	3.4	0.55	0.1	0	23.8
Command	Celery	3	3.4	0.99	0	0.2	24.3
Command	Celery	2	3.4	0.80	0.3	0.1	20.8
A0160	*A.panul*-Wild	8	3.5	.	0.4	5	10.5
UC12A45	Celery	2	3.5	.	0.2	0	24.0
UC12A45	Celery	3	4.5	0.76	0.6	0	23.3
A0012	Celeriac	3	3.6	.	0.3	0	17.2
A0014	Celeriac	6	3.6	0.37	0.1	0	14.8
A0061	Feral Celery	3	3.6	0.62	0.1	0	18.8
A0230	*A.prostratum*-Wild	8	3.6	1.04	0.1	0	7.6
A0078	Celery	6	3.8	0.39	0.2	0	13.6
A0150	Celeriac	6	3.8	0.70	0.2	0.2	20.6
A0180	Celeriac	6	4	0.20	0.2	0	18.2
A0068	Smallage	6	4.2	0.56	0.4	0	19.6
A0135	Smallage	6	4.3	.	0.5	0	17.2
A0069	Smallage	6	4.4	.	0.5	0	17.8
A0101	Celeriac	3	4.4	.	0.5	0	18.8
A0330	Celery	6	4.5	0.43	0.5	0	14.4
A0015	Annual Smallage	6	4.6	0.39	0.8	0.6	22.2
A0142	Annual Smallage	6	4.8	.	0.8	0	9.3
A0229	*A.annum*-Wild	8	5	.	1	0.8	0.0

Table 4. History of accessions selected for field testing against race 4

Acc.#	*Species*	Crop	Origin	Origin al Acc.#	Variety
A0165	*A. graveolens var. graveolens ?*	Celeriac	Busambra , Palermo , Italy	72, U. of P.	unknown
A0235	*A. graveolens*	unknown	China	124	unknown
A0163	*A. graveolens var. dulce*	Celery	Stone River , Sinkiang , China	Orton 0111	Local type
A0103	*A. graveolens var. secalinum*	Smallage	Turkey	PI165064	unknown
A0021	*A. graveolens var. rapaceum*	Celeriac	Yugoslavia	PI357327	Domasen
A0102	*A. graveolens var. rapaceum*	Celeriac	Turkey	PI164944	unknown

Objective 2. Next generation sequencing of 15 isolates so that we can select race-specfic primers for rapid PCR detection of race 4 and for quantification of inoculum levels in soil, particularly of race 4.
We selected 15 isolates: six race 2 isolates selected over time and location; one race 4 isolate, six of the polymorphic non-pathogenic "clade 5" isolates that we detect in celery with Fusarium yellows but that don't cause symptoms; and two of the polymorphic non-pathogenic clade 1 isolates that we less frequently detect in plants with Fusarium yellows, but that don't cause symptoms. Illumina Mi-Seq was used to generate 10X coverage with approximately 200 bp paired-end reads. We did both a *de novo* assembly and an assembly against the best assembled, full genome sequence that is available from the Broad Institue: *F. oxysporum*: strain 4287 which is a f. sp. *lycopersici* (tomato) race 2. 65,000 single nucleotide polymorphisms were identified and used to compare the strains by Nei's distance (Table 3). Based on a comparison of each strain to the reference 4287 strain, we identified 1 kb regions that are present at 10X or more coverage in only (and in all the) the race 2 isolates and in only race 4. Regions of interest were examined with the software CLC. Approximately 20 potential primers, for each of just race 2 and for just race 4 were designed using Primer3, and blasted at NCBI; many of these primers appeared to be specific to *Foa* race. Next, the presumed amplicons were blasted at the Broad comparative Fusarium database. Here, we found that the primer pairs would amplify typically two or three of the full-genome *Fusarium oxysporum* reference strains that were sequenced at the Broad Institute. As a proof a concept, we selected one of the race 4 primers and demonstrated that it only amplified race 4 from representatives of our collection. To summarize, we have primers that we could use to rapidly identify race 4 from plants and to quantify growth of race 4 vs. race 2 vs. the isolates that infect but don't cause symptoms. However, we know that these primers will amplify some *Fusarium oxysporum* that are present in soil. Consequently, we propose to continue this objective for one more year to find sequences that are present in only race 4 and in only race 2 and that are not present in any of the full genome-sequenced strains at the Broad.

Objective 3. Development of a DNA assay for determination of (*Foa*) inoculum concentration in soil. We have a quantitative PCR assay that appears to adequately remove PCR inhibitors from California celery soil.

Objective 4. Optimization of an assay for response to *Foa*
Table 6 shows our results from several trials with *Foa* race 2. Last year, we hoped that a seedling assay would allow us to more rapidly assess resistance (and virulence). However, this past year we discovered that non-pathogenic isolates also cause stunting of celery seedlings.

Objective 5. Differentiation of resistance to Foa race 2 from *Fu1* and other genes.
Race 2 resistance in UCD-derived material is currently dependent on major resistance gene *Fu1* and a couple of minor genes; none of the UCD-derived cultivars have more resistance than Challenger and some such as Command have less resistance. "Stacking" resistance from multiple sources will require either some ability to see greater resistance than in Challenger or some markers for resistance; we currently have neither.

This past year, we had a visiting student that screened potential markers for the *Fu1* resistance; we currently have one candidate (Fig. 4) that can be screened against a segregating population for tolerance vs. susceptibility.

Table 5.

Nei's distance between strains based on 65,000 single nucleotide polymorphisms: 0, identical; 1, different)

		Race 2						(NP, Non-pathogenic)						Race 1?	NP	Race 4
	Strains	3	67	207.A	226.2A	247.1A	258.1B	241.1A	221.B	223.1A	226.1A	226.1B	261.1A	268.2	270.A	274.AC
RACE 2	3															
	67	0.0027														
	207.A	0.0022	0.0022													
	226.2A	0.0025	0.0025	0.0022												
	247.1A	0.0026	0.0026	0.0022	0.0025											
	258.1B	0.0022	0.0023	0.0017	0.0021	0.0023										
(NP)	241.1A	0.29	0.29	0.29	0.29	0.29	0.29									
	221.B	0.31	0.31	0.31	0.31	0.31	0.31	0.16								
	223.1A	0.30	0.30	0.30	0.30	0.30	0.30	0.16	0.12							
	226.1A	0.30	0.30	0.30	0.30	0.30	0.30	0.16	0.15	0.12						
	226.1B	0.31	0.31	0.31	0.31	0.31	0.31	0.16	0.12	0.12	0.14					
	261.1A	0.30	0.30	0.30	0.30	0.30	0.30	0.16	0.09	0.11	0.14	0.12				
Race 1?	268.2	0.59	0.59	0.59	0.59	0.59	0.59	0.66	0.68	0.67	0.66	0.68	0.67			
NP	270.A	0.62	0.62	0.62	0.62	0.62	0.62	0.69	0.71	0.71	0.70	0.71	0.70	0.30		
RACE 4	274.AC	0.61	0.61	0.61	0.61	0.61	0.61	0.68	0.70	0.69	0.68	0.70	0.69	0.06	0.31	

Fig. 4.

A simple sequence repeat PCR marker (ECMS 39) possibly associated with resistance to *Foa* race 2

Primer sequence from Wang et al. 2011. Genetic diversity in *Apium graveolens* and related species revealed by SRAP and SSR markers. Scientia Horticulturae 129:1–8.

Table 6. Performance of selected accessions in the greenhouse assay against *F. oxysporum* f. sp. *apii* race 2. Ten plants were assayed per replicated per trial for both the race 2-inoculated and mock-inoculated.

Cultivar	Crop	Trial	Avg. vascular discoloration rating, 0 (none) to 5	Fraction of height of mock-inoculated controls, excluding dead plants	Fraction of plants that died	**Mock-inoculated controls**: avg. vascular discoloration rating, 0 (none) to 5	Avg aerial height of plant, cm
A0102	Celeriac	3	0	0.75	0	0	28.0
A0112	Celeriac	1	0	0.95	0	0	34.5
A0012	Celeriac	1	0.1	1.03	0	0.3	31.8
A0112	Celeriac	3	0.4	0.75	0	0	28.4
A0012	Celeriac	3	0.2	1.10	0	0	17.7
A0235	Unknown	3	0	0.82	0	0	35.4
A0027	Celeriac	1	0.2	1.00	0	0.4	30.3
A0140	Celeriac	1	0.2	1.25	0	0.1	32.9
A0169	Celeriac	3	0.2	1.15	0	0	25.1
A0173	Celeriac	3	0.2	0.93	0	0.1	26.6
A0210	Celeriac	1	0.2	0.98	0	0.3	29.7
Challenger	Celery	3	0.2	0.70	0	0	28.9
Challenger	Celery	1	0.3	0.88	0	0.1	31.1
Sabroso	Celery	3	0.2	0.95	0	0	28.9
Sabroso	Celery	1	0.6	1.05	0	0	31.6
UC390S-2	Celery	3	0.2	1.14	0	0	31.7
UC390S-2	Celery	1	0.7	0.85	0	0	31.3
A0136	Celeriac	1	0.3	1.08	0	0.3	32.5
A0127	Celeriac	1	0.5	0.87	0	0	30.6
Command	Celery	3	0.5	1.10	0	0.2	26.6
Command	Celery	1	1.6	1.00	0	0.2	29.1
A0146	Celeriac	1	0.8	0.87	0	0.2	31.8
UC12A45	Celery	1	0.8	1.15	0	0	30.8
UC12A45	Celery	3	1.1	0.89	0	0	30.1
Tall Utah 52-70R improved	Celery	3	1	0.99	0	0	31.4
Tall Utah 52-70R improved	Celery	1	3.1	0.59	0.2	0.1	22.3
UC040A	Celery	1	1.2	0.93	0	0.1	30.7
A0061	Feral Celery	3	1.3	0.81	0	0	28.4
A0165	Celeriac	3	1.3	0.84	0	0.3	21.3
A0101	Celeriac	3	4.5	.	0.7	0	16.2