2023-24 Annual Report

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RESEARCH PRIORITIES

Production	✓	Evaluate insect management tools, alternatives to organophosphates and carbamates (especially products which are subject to restriction or loss due to FQPA and other regulations - e.g., acephate, diazinon, carbamates)
	✓	Study the biology and management of soil pests
	✓	Study management of Sclerotinia (airborne and soil borne)
	✓	Evaluate new celery varieties for resistance to insects and diseases
	✓	Evaluate biofumigants as tools for pest control in celery
	✓	Develop resistance management strategies for all pest categories
	✓	Continue weed control research to find complimentary or replacement products for Lorox/linuron Caparol/prometry and Caparol/Prometryn
	✓	Evaluate the secondary effect of loss of methyl bromide as a tool used in rotational crops on celery production areas
Regulatory	✓	Registrants should insure that air and chemigation labels are a part of all new product registrations
	✓	Address REI issues/concerns for products used in late season (e.g. aphicides)
	✓	Multiple products should be allowed under 24(c) and Section 18's for resistance management
	✓	Expedite registration of Dual Magnum/S-metolachlor for nutsedge control
Educational	✓	Educate regulators, legislators, and policy makers on need for more than one product for a particular pest for effective resistance management
	✓	Educate regulators on what a suitable replacement product is and what constitutes commercially acceptable levels of control
	✓	Educate growers, PCAs, agencies and the urban community on the relationship of waterway management to weed and insect pests
	✓	Educate the urban population about agricultural practices necessary for celery production
	✓	Educate the public on the nutritional values of California grown celery and the high level of food quality standards established for this commodity

CALIFORNIA CELERY RESEARCH BOARD

September 1, 2023 through August 31, 2026

DIST	MEMBER	ALTERNATE
2	**Adrian Zendejas** Desert Mist Farms	**VACANT**

DIST	MEMBER	ALTERNATE
3	**Hank Laubacher Jr** Laubacher Farms	**Erik Heacox** Tanimura & Antle
3	**Geremy Olsen** Deardorff Family Farms	**Jackson Duda** Duda Farm Fresh
3	**Danny Pereira – Chairman** Rio Farms - **RES COMM**	**Bryson Daniel** Boskovich Farms
3	**Steve Adams** Boskovich Farms - **RES COMM**	**Ryan Coultas** Coultas Vegetable
3	**Mike Naumann - Vice & Research Chair** Naumann Ranch - **RES COMM**	**Will Terry** Terry Farms

DIST	MEMBER	ALTERNATE
4	**Philip Adam** Innovative Produce	**VACANT**
4	**Tyler Grossini** Campbell Ranches	**Matt Hamon** Campbell Ranches

DIST	MEMBER	ALTERNATE
5	**Alejandro Palma** Dole Fresh Vegetable	**Larry Knerr** Duda Farm Fresh
5	**Greg Lewis - RES COMM** Duda Farm Fresh	**Julia Harshman - RES COMM** Duda Farm Fresh
5	**Kevin Brink - RES COMM** Ocean Mist Farms	**Phillip Montagna** Church Brothers

PUBLIC MEMBER
Steve Donovan - RES COMM

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CELERY RESEARCH ADVISORY BOARD
FISCAL YEAR ENDING 9/30/24
September 30, 2025 (FINAL)

INCOME		CARRYOVER	344,185	
		BUDGET	YTD	BALANCE
30-110	Current	270,000	303,332	(33,332)
30-111	Prior	-	-	-
30-120	Interest Income	8,000	13,332	(5,332)
30-140	Other Income	-		-
TOTAL INCOME		**278,000**	**316,665**	**(38,665)**

EXPENSES				
30-305	Management Services	58,320	58,320	-
30-315	Audits	5,000	5,300	(300)
30-320	Office Supplies	600	629	(29)
30-330	Telephone	1,000	812	188
30-335	Postage	400	500	(100)
30-355	Travel & Mileage	1,500	924	576
30-360	Meetings	2,500	1,637	863
30-365	Annual Report	50	-	50
30-370	Insurance	1,250	877	373
30-390	Miscellaneous	100	-	100
30-392	Website	500	362	138
30-513	Research Reserve	7,000	7,000	-
30-515	CA Specialty Crops Council	6,000	6,000	-
30-530	No Cost Extension	51,483	20,210	31,273
30-551	Production Research - UC Davis	68,723	66,171	2,552
30-552	Production Research - UC Riverside	75,630	30,114	45,516
30-554	Production Research - UCCE	16,354	6,283	10,071
30-556	Production Research - Outside	52,979	50,479	2,500
30-710	Marketing Branch	21,500	25,836	(4,336)
30-711	Market Enforcement Branch	2,500	-	2,500
TOTAL EXPENSES		**373,389**	**281,451**	**91,938**

NET INCOME	**$**	**35,214**
CASH BALANCE	**$**	**379,399**

NO COST EXTENSIONS - TO BE PAID	**$**	**31,273**
CASH BALANCE - AFTER NO COST EXTENSIONS PAID	**$**	**348,126**

Research Project Worksheet
2023-24

RESEARCHER	PROJECT TITLE	COMMITTEE REC	BOARD Approved 9-20-23
Lynn Epstein *UC Davis*	**Genomic Tools for Breeding for Resistance in Celery to F. oxysporum f. sp. apii (Foa) Races 4 and 2**	**68,723**	**68,723**
Renee Eriksen *USDA - Salinas*	**Celery Breeding for Resistance to Fusarium Oxysporum f. sp. apii Race 2 and 4**	**64,463**	**64,463**
	Eriksen - USDA	*27,979*	
	Daugovish - UCCE ANR Ventura	*9,373*	
	Greer - UCCE ANR San Luis Obispo	*6,981*	
	Putman - UC Riverside	*20,130*	
Nicholas LeBlanc *USDA - Salinas*	**Diagnostic Test for Identification and Quantification of Fusarium Oxysporum f. sp. apii Race 4 in Soil and Plant Material**	**25,000**	**25,000**
Chow-Yang Lee/Kund *UC Riverside*	**Integrated Pest Management on Celery**	**55,500**	**55,500**
Perring/Chow-Yang Lee *UC Riverside*	**Insecticide Resistance of Celery Pests Focusing on the Lygus Bug, Lygus Hesperus**	*(funded $15,000 during the previous year)*	
CSCC	**California Specialty Crops Council** (Annual Fee)	*Take to Board*	**6,000**
	TOTAL REQUEST	**213,686**	**$219,686**

Layperson's summary

CALIFORNIA CELERY RESEARCH ADVISORY BOARD
RESEARCH PROJECT REPORT

for the period of:
October 1, 2023 - September 30, 2024

PROJECT TITLE: Genomic tools for breeding for resistance in celery to *F. oxysporum* f. sp. *apii* (*Foa*) races 4 and 2

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

COOPERATING PERSONNEL: Chaehee Lee, Sukhwinder Kaur, Peter Henry, Grey Monroe, and Allen Van Deynze

Genetic resistance is the best method for control of pathogens in the *Fusarium oxysporum* species complex. The *F. oxysporum* strain that causes disease on celery is called *F. oxysporum* f. sp. *apii* (*Foa*). Celery grown in infested areas in California can succumb to two different, and distantly related *Foa*: *Foa* race 2, which was first reported in 1976 (Hart and Endo, 1978), and *Foa* race 4, which was first detected in Camarillo in Ventura County in 2013 (Epstein *et al.*, 2017). Before our recent research program, the only assembled genome of celery that was publicly available was cv. Ventura (Song *et al.*, 2021); but very few of the Ventura "gene models" have been publicly annotated, and this limits the utility of this genome. Also, Ventura is completely susceptible to *Foa* race 4, and while Ventura is more resistant than Tall Utah types to *Foa* race 2, Ventura is what is now considered to be susceptible to *Foa* race 2 (Kaur and Epstein, unpublished).

In the 1980's, UC Davis (UCD) researchers identified *Apium graveolens* accessions that were resistant to *Foa* race 2; after initial breeding at UCD (Orton *et al.*, 1984), the celery cultivar Challenger was developed in the private sector by Rob Pybus. More recently, we used Challenger as the celery parent for the cross with USDA PI 181714 for race 4 resistance (Epstein and Kaur, 2023). We then selfed the F1 three consecutive times with selection for both resistance and celery-type; resistance to both *Foa* race 4 and race 2 appear to be fixed in the line that we used here (F1S3 76-8-36-124), which we call "F1S3" in this report.

Here, we report the preparation and submission of a publication on the Challenger genome. The genome is very well assembled into 11 chromosomes that were "anchored" with a genetic map. With publication, the following celery genomic resources will be available, for the first time, to the entire celery breeding community: the identification of the DNA sequences (in both coding and non-coding regions) of 40,464 protein coding genes with as much functional information as the bioinformatics allows; and the DNA sequence of genes that only encode for RNA, including 129 microRNAs, which might be relevant for disease interactions. Most of the major resistance (R) genes, including those that encode for resistance in other *F. oxysporum*-plant interactions, are in two major families: the nucleotide-binding and leucine-rich repeat receptors (NLR) and pattern recognition receptors (PRR). Here document that 243 NLRs and 445 PRRs are expressed in Challenger's crowns, a critical site for resistance to *Foa* race 2. While the final steps on the F1S3 genome and resistance gene candidates to *Foa* race 4 are still in progress, here, we also summarize our progress since our last report on F1S3.

CALIFORNIA CELERY RESEARCH ADVISORY BOARD
RESEARCH PROJECT REPORT

for the period of:
October 1, 2023 - September 30, 2024

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

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OBJECTIVES**:**

Objective 1. To provide the celery breeding community with the most useful genomic tools, including a publicly available, fully annotated, chromosome-level genome of cv. Challenger, along with a genome-by-sequencing (GBS) protocol and markers.

Objective 2. To produce a high-quality assembly and annotation of a celery breeding line (F1S3 76-8-36-124) which is resistant to *Fusarium oxysporum* f. sp. *apii* (*Foa*) races 4 and 2. The specific sub-objectives are:

- 2a. To assemble a high-quality chromosome-level genome of the race 4-resistant celery breeding line (F1S3 76-8-36-124), which is fixed (=homozygous) for resistance to *Fusarium oxysporum* f. sp. *apii* race 4;
- 2b. To collect and use Iso-Seq data (full length transcripts) to annotate the genes in F1S3 76-8-36-124 crowns and roots under race 4 disease pressure;
- 2c. To collect and use Tag-Seq (=RNA-Seq) data of F1S3 76-8-36-124 race 4-resistant vs. Challenger (race 4-susceptible) to i) identify candidate resistance and susceptibility genes in the breeding line and in celery, respectively, ii) to identify candidate "effector" genes in *Foa* race 4 that affect the outcome of the host-pathogen interaction and iii) to help understand the "downstream" pathways that result in either resistance or susceptibility;
- 2d. To use all the available datasets (including the Illumina sequence from bulk segregant analysis of race 4-resistant vs. susceptible populations, the F1S3 assembly from Objectivs 2a and 2b, and the Challenger genome assembly) to identify candidate genes that confer resistance to *Foa* race 4 in celery.

RESULTS: The Tables and Figures below show either new, or when indicated, more recent analyses than were shown in previous reports.

Objective 1. To provide the celery breeding community with the most useful genomic tools, including a publicly available, fully annotated, chromosome-level genome of cv. Challenger, along with a genome-by-sequencing (GBS) protocol and markers.

We have prepared a publication on the Challenger genome that is in the review process. In that manuscript, we provide the DNA sequence of Challenger on each of the 11 chromosomes, and, most importantly, the amino acid sequence of the 40,464 protein-coding gene models. Main Table 1 summarizes details about the final assembly. Some additional details about the assembly are in last year's report.

Main Table 1. Descriptive statistics about the *Apium graveolens* cv. Challenger genome and annotation.

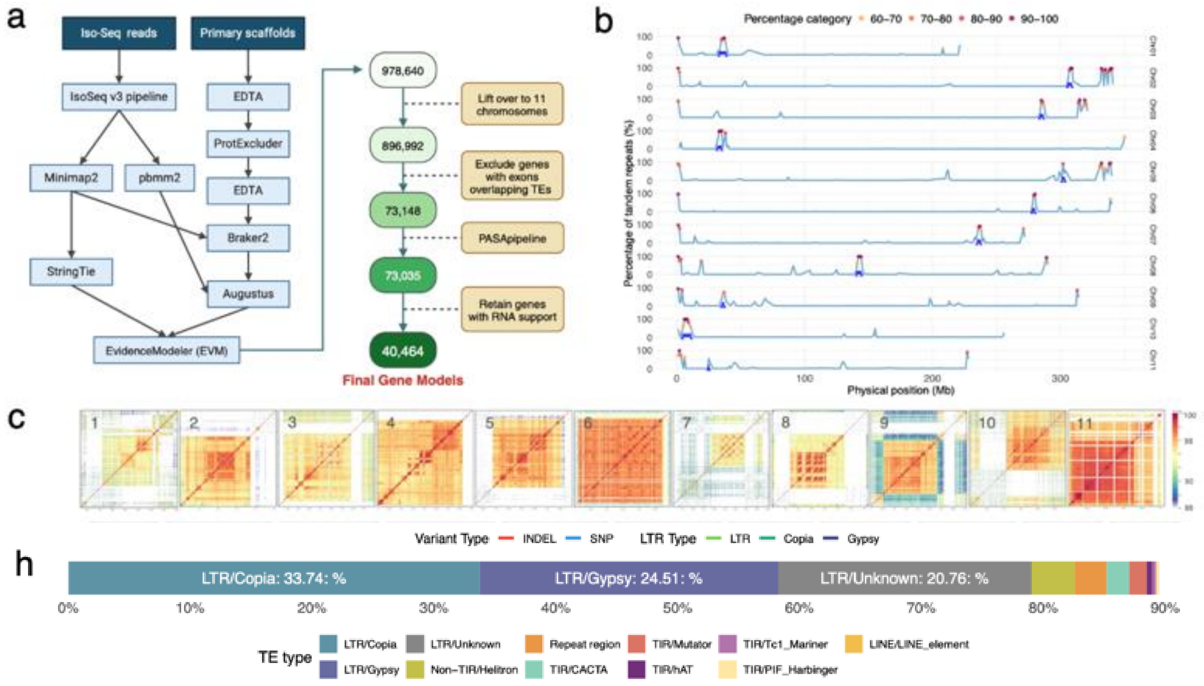
	***A. graveolens* 'Challenger'**	
Genome assembly	**Primary assembly**	**Chromosomes**
Number of Sequences	2,253	11
Size (bp)	3,428,536,321	3,267,747,404
Max length (bp)	303,905,413	349,664,189
N50 (bp)	162,067,254	320,415,245
L50	8	5
L90	32	10
Number of Gaps		52
%GC	36.6	35.7
BUSCO completeness (%)	98.6	98.6
complete single-copy (%)	91.6	93.4
complete duplicated (%)	7.0	5.1

Protein-coding gene	**Chromosomes**
No. of genes	40,464
Total length of genes (bp)	154,876,985
% of genome covered by genes	5
Mean gene length (bp)	3,827
Mean CDS length (bp)	1,231
Mean exon length (bp)	279
Mean intron length (bp)	539
Mean exon number per gene	5
BUSCO completeness (%)	97.3
complete single-copy (%)	92.8
complete duplicated (%)	4.6

RNA loci	**Number**	**Total (bp)**
tRNA	1,000	76,298
rRNA	4,292	1,274,972
miRNA	129	16,314
snoRNA	12,115	1,285,805

We note that high quality "annotation" of the amino acid sequence is particularly important for genome-assisted celery breeding because only 5% of the celery DNA encodes for proteins, and on average, each celery gene has five coding regions (called exons) and 4 interspersed non-coding regions (called introns). In celery, over 86% of the genome is comprised of repetitive elements, which used to be called "junk DNA;" the large quantity of "junk DNA" in celery increases the amount of work that we have to do at almost every step of our analyses.

Below, we show a part of a publication figure that wasn't in our previous report. Main Fig. 1a has the workflow for the identification of the 40,464 protein-coding gene models. Fig. 1b has information on the position of some repeat regions -- the centromeres and the telomeres. Fig. 1c shows evidence for assignment of the centromere positions. Identification of the centromeres is important for understanding the recombination data that we show below and identification of telomeres is important for determining whether the entire chromosome (end to end) has been sequenced. Fig. 1h has information on the composition of transposable elements in Challenger.



Main Fig. 1. Genomic features and annotation of the *Apium graveolens* celery cv. Challenger genome. **a** Overview of the genome annotation workflow with the selection of 40,464 gene models. **b** Percentage of tandem repeats per 1 Mb window across chromosomes ranging from 0-100% on the y-axis. The windows with different percent ranges > 60% are marked in different colors. The blue triangles indicate the start and end positions of potential centromeres, highlighted by blue boxes. **c** Identity heatmaps displaying patterns of sequence identity of the centromeric region for each chromosome. **h** The major groups of transposable elements and repeat DNA in Challenger, from groups that account for the most bp to the least; LTR, long terminal repeats; TIR, terminal inverted repeats of transposable elements; and LINE, long interspersed nuclear elements.

Supplemental Table S6. The percentage of length, genes, LTRs, BUSCO genes, NLRs, and PRRs in the low-recombination regions of each chromosome[a]

	Percentage in the low recombining region					
Chr	Chromosome length[b]	Genes	LTRs	BUSCOs	NLRs[c]	PRRs
Chr01	81.2	59.3	85.9	57.1	-	36.8
Chr02	83.8	61.8	89.1	60.9	-	50
Chr03	86.7	67.1	91.1	68.4	-	75
Chr04	87.5	61.8	91.9	63.9	-	54.4
Chr05	83.8	64.6	89	52.9	-	63.6
Chr06	67.3	47.4	70.4	50.3	-	26.7
Chr07	83.2	64.2	88.2	61.7	-	49.2
Chr08	83	61.9	88.4	70.8	-	35.7
Chr09	72	46.7	76.7	49.7	-	52.6
Chr10[d]	55.3	25.5	60.1	21.4	-	12.8
Chr11	81.2	59	86.7	62.5	-	50
(Entire Genome)		56	83.7	55.5	15.7	45.5
	(n)	(40464)	(2.68 million)	(1497)	(287)	(494)

[a] Based on the genetic map as a function of the physical map, the region that has the longest line with a slope of <0.025 cM/Mb that had no points 2 cM from the line. Only cells with more than 10 counts are evaluated. A 2 x 2 chi-square analysis of the counts in the lower-recombination regions versus the higher recombination region across the entire genome for genes versus BUSCOs indicated no significant differences in their distribution ($P=0.69$). In contrast, LTRs were significantly more concentrated in the lower-recombination regions than in the higher recombination regions (ChiSquare Likelihood ratio=16832, $P<0.0001$), and both NLRs (ChiSquare Likelihood ratio= 157) and PRRs (ChiSquare Likelihood ratio= 22) were highly significantly more concentrated in the higher-recombination regions ($P<0.0001$).

[b] Based on the length of the pseudochromosome.

[c] Only 16% of the NLRs are in the lower recombination regions, and no chromosome had more than 9 counts in those regions.

[d] Chromosome 10 is telocentric and consequently has no recombination region on one side of the chromosome.

Supplemental Table S4. The genetic and physical maps of *Apium graveolens*: recombination rates (RR) across the pseudochromosomes[a]

	Entire pseudochromosome						Low recombination region on the pseudochromosome[c]				
Chr	No. markers	No. Bins	Total (cM)	Length (Mb)[b]	cM/Mb	No. genes	Start (Mb)	End (Mb)	% of total length	% of total cM	% of total no. of genes
Chr01	647	158	77.5	215.1	0.36	2956	12.5	192.3	83.6	7.9	59.3
Chr02	427	176	129.5	334.8	0.39	3972	37.2	322.3	85.2	2.9	61.8
Chr03	425	137	121	318.4	0.38	3852	20.4	298.1	87.2	6.8	67.1
Chr04	390	143	120.4	345	0.35	4345	17.8	323.8	88.7	8.7	61.8
Chr05	565	191	112.9	334.1	0.34	3945	24.1	308.5	85.1	6.7	64.6
Chr06	474	169	120.6	335.4	0.36	4262	60.7	289	68.1	5	47.4
Chr07	281	136	110	267.6	0.41	3479	21.9	247.7	84.4	7.7	64.2
Chr08	398	156	116.8	285.2	0.41	3259	17.8	258.1	84.3	5	61.9
Chr09	640	174	117.2	310.1	0.38	3762	28.4	254	72.8	3.1	46.7
Chr10	588	165	83.4	250.4	0.33	3870	3.4	144.7	56.4	3.9	25.5[d]
Chr11	426	120	83.3	222.8	0.37	2762	12.6	197.4	83	2.6	59

[a] There were 11 linkage groups, which corresponded to the eleven chromosomes identified in Song et al. (Plant Biotech. 2021; 19:731).

[b] The entire pseudochromosome is defined as the length of the physical distance from a marker in the first bin to one in the last bin,.

[c] Using the curves shown in the Fig. 1d genetic map as a function of the physical map, we selected the longest line with a slope of <0.025 cM/Mb that had no points 2 cM from the line.

[d] Chromosome 10 is telocentric.

Main Fig. 2. Celery cv. Challenger's nucleotide-binding (NB) leucine-rich repeat receptors (LRRs) (NLRs). **a** Diagram of Challenger's 11 chromosomes: gene density, boundaries of the lower recombination region and location of 287 NLRs. Inside each chromosome, gene density is shown on a scale from white (0 genes/Mb) to red (97 genes/Mb). Above each chromosome, the region between the two arrows and the extending dark line is the lower-recombination region. The symbols below the chromosomes indicate the locations of the 287 NLRs. Purple squares indicate NLR clusters with 3 or more NLRs with no more than two non-NLR genes among the NLR genes within the cluster. **b** Distributions of DESeq2-normalized counts of 243 NLRs expressed in Challenger crowns in a mock-infested treatment: 90 CC-NB-LRR (CNL); 42 NB-LRRs without a 5' domain (NL); 5 RPW8-NB-LRR (RNL); and 106 TIR-NB-LRR (TNL). Groups are based on the similarity of Ventura's homologous DNA to that of Challenger: identical in 100% of the length; highly similar, <100≥98% identical in 98% of the length; somewhat similar, <98≥75% identical in 75% of the length; and highly dissimilar, <75% identical in 75% of the length. Asterisks show the selections in part c. **c** DESeq2-normalized expression levels with the 95% confidence interval of three selected NLRs in celery crowns in *Foa* race 2-resistant Challenger, and in a separate experiment, race 2-susceptible Tall Utah 52-70R Improved (TU). After the plants were mock-infested or infested with either *Foa* race 2 or *Foa* race 4, the Challenger crowns were harvested at the indicated time points and the TU were harvested at 21 dpi. The selections are in the upper quartile of expression of all NLRs, their DNA differs from that of their homologs in Ventura, and the homolog was not either not expressed or negibily expressed in TU: chromosome 4 NLR 69 (gene AgCh.04.g150590); chromosome 6 NLR 18 (AgCh.06.g193140); and chromosome 4 NLR 56 (AgCh.04.g150320).

Because we have a genetic map of *Apium graveolens*, we were able to examine the recombination rates of genes that are in different positions along the chromosome. That is, with 40,464 genes in 11 chromosomes, there are an average of 3,679 genes per chromosome (Supplemental Table S4 below). As shown in last year's report's comparison of the *A. graveolens* genetic map with Challenger's "physical map," celery has a two very different "recombination landscapes," with a considerably long region in the middle of each chromosome that has a very low recombination rate. A comparison of the expressed SNP (eSNP) markers on the genetic map and the physical map revealed that for 8 of the 11 chromosomes, 83 to 89% of the length of the chromosome has a reduced recombination rate of <0.025 cM/Mb. In contrast, in the ends of the arms of the chromosomes, there is an average recombination of 2.0 ± 0.2 cM/MB/chromosome for the 11 chromosomes. Not surprisingly, there is a lower gene density in the region with a reduced recombination rate (Main Fig. 1a). However, the regions with lower recombination rates still have 56% of the genes (Supplemental Table S4). While comparatively infrequent crossovers are expected in the region of the centromere, the regions of lower recombination, as defined here as <0.025 cM/Mb, extend well beyond the centromeric region in Challenger to 56% of the length of the entire chromosome in the telocentric Chromosome number 10 to a range from 68% to 89% of the lengths of the nine subtelocentric pseudochromosomes and 84% of the length of metacentric Chromosome number 8 (Supplemental Table S4). Thus, to summarize, slightly more than half of the celery genes are in a very low recombination zones. This is likely to have breeding implications, particularly if there are genes from the wilder resistant parent that are linked to the desired resistance gene from the resistant parent.

In celery, different categories of genes tend to have different distributions. In Supplemental Table S6, we show the distributions in low recombination vs. higher recombination zones in several groups: all 40,464 protein-encoding genes; LTR, the non-coding classic "junk" DNA in genomes; BUSCO, highly conserved and thus essential genes in plants that are typically tracked in plants for quality control of the genome assembly; NLRs, which are a group of genes that are a key part of plants' early-response to pathogens; and PRRs, which include critical genes that are part of plants' surveillance system for pathogens. Importantly, the NLRs are almost all in the higher recombination zones and in clusters, as discussed below.

In the absence of pathogen pressure, historical plant breeding for maximal yield can lead to the loss of the full arsenal of genes that encode for pathogen surveillance, i.e., the NLRs and the PRRs (Barragan and Weigel, 2021). The NLRs are part of effector triggered immunity (ETI), An early version of Main Fig. 2a was shown in last year's report, but there are a number of improvements in the version shown below. Main Fig. 2a shows chromosomes (Chr) 01 – 11. Inside each chromosome, there's a representation of the gene density of 0 genes per million nucleotides in white to deep red with 97 genes per million nucleotides. The twist in each chromosome shows the location of the centromere –genes in centromeric regions are expected to have a lower recombination rate. But the lower recombination rate in celery (shown in Supplemental Table S4), extends further than expected, as shown by the dark lines and downward pointing arrows on each chromosome in Main Fig. 2a. We identified 287 individual NLRs in Challenger, and quantified expression of 243 NLRs in crown tissue. The positions of the 287 NLRs in Challenger are shown under each chromosome as purple squares for clusters when there are three or more, which account for 58% of the NLRs, or as green circles when there are pairs of NLRs, which account for 15% of the NLRs, or as orange triangles when a NLR is not adjacent to another NLR (a singleton). Chromosomes 4, 7 and 11 each have a "multicluster region" near the chromosome terminus, in the higher recombination region. Chromosome 4 has a 16 Mb multicluster with 60 NLRs, chromosome 7 has a 0.8 Mb multicluster with 25 NLRs and chromosome 11 has a 11 MB multicluster with 62 NLRs. Together, the three multiclusters account for 51% of the 287 NLRs. Overall, 242 (84%) of Challenger's NLRs are in the higher recombination regions on the ends of the chromosomes (Supplemental Table S6).

NLRs can be sub-classified into types: CNLs and TNLs generally serve as "sensor" NLRs; and RNLs and NLs as "helper" NLRs (Supplemental Fig. S5). Because NLRs are such an important source of iconic Resistance (R) genes in other plant-pathogen interactions, including in other plant-*F. oxysporum* interactions, we wanted to get an inventory of NLRs in Challenger, which was the susceptible parent in the F1S3 in Objective 2.

In addition, although we don't have a biological experiment that will allow us to conclusively identify resistance gene(s) to *Foa* race 2 in Challenger (as we do with the Bulk Segregant Analysis for resistance to *Foa* race 4 in F1S3), we now have bioinformatic tools to at least investigate hypotheses about resistance to *Foa* race 2 in Challenger. Consequently, we asked whether there were important differences in the NLRs in Challenger, which is resistant to *Foa* race 2, and Ventura, which is moderately susceptible to *Foa* race 2. We asked this question in three somewhat independent ways. First, we compared Challenger and Ventura (Main Table 2); a preliminary analysis was shown in last year's report. Here, using the 116 TNLs, 107 CNLs, 5 RNLs and 59 NLs that we documented in the Challenger genome (Table 2), we used BLASTn to identify homologs in the Ventura genome. Among the 287 total NLRs, while Ventura and Challenger had identical DNA sequences over 100% of the length of the gene model in 141 (49%) NLRs, Ventura had 63 (22%) with <75% identity over at least 75% of the length of the Challenger model. An additional 10% of Ventura's NLRs had between 75% and 98% identity in at least 75% of the length of the Challenger model. The point is that the DNA sequence of the NLRs in Challenger and Ventura differ, and consequently, Ventura may have mutations that have inactivated the gene that encodes for *Foa* race 2 resistance. This hypothesis is at least somewhat corroborated by the result that in the essential, highly conserved BUSCO genes, Ventura and Challenger's DNA sequences are more similar than with the NLRs, which can be considered to be non-essential genes, particularly in situations in which there is no disease pressure and no selection for retention of a NLR which might confer resistance to *Foa* race 2 (Supplemental Table S9).

Second, we used the software NLR-Annotator to count NLRs in the DNA assemblies of the two cultivars. While Challenger had 119 TNL, Ventura had 14 fewer (Supplemental Table S10). Similarly, Challenger had 110 CNL, whereas Ventura had 21 fewer. Notably, these differences were particularly apparent in the TNL-dominated NLR multicluster on chromosome 4 and the CNL-dominated NLR multicluster on chromosome 7. To summarize, these results support the hypothesis that Ventura, as a representative of celery cultivars, has lost some NLRs that were present in the resistant progenitor of Challenger.

Supplemental Fig. S5

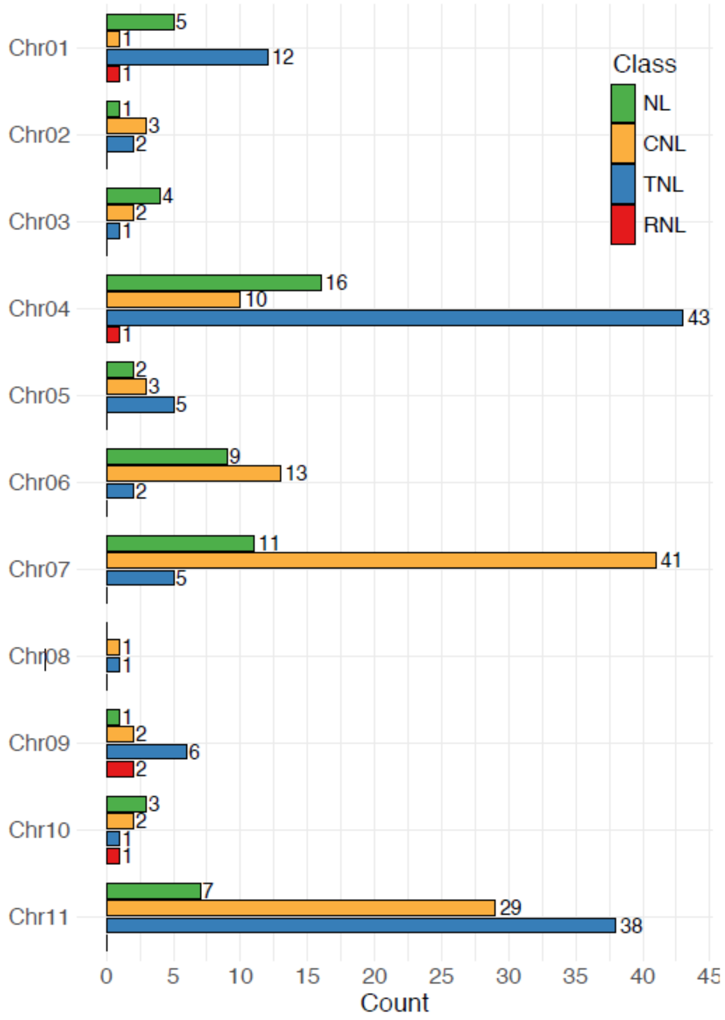


Fig. S5. The number of Challenger's 287 NLRs in each chromosome by NLR type.

Main Table 2. Similarity of NLR homologs between cv. Ventura and Challenger[a]

Category of similarity between Challenger NLRs in Ventura	Criterion for category: identity (and length of alignment), %[a]	No. in each category (Expressed in Challenger, %[b])			
		TNLs	CNLs	RNLs	NL
Identical	100 (100)	59 (90)	51 (94)	3 (100)	28 (61)
Highly similar	<100≥98 (98)	21 (86)	16 (94)	2 (100)	15 (80)
Somewhat similar	<98≥75, (75)	4 (100)	19 (89)	-	6 (100)
Highly dissimilar	<75, (75)	32 (97)	21 (95)	-	10 (80)
Total		116 (91)	107 (93)	5 (100)	59 (73)

[a] Two hundred eighty-seven NLR loci in Challenger were manually confirmed as either in a bioinformatically annotated gene (n=195) or in an open reading frame (ORF) of a locus (n=92). Two hundred forty-three NLRs were expressed in crown tissue, and an additional 11 were expressed in other tissues.

[b] TNL, Toll/Interleukin-1 receptor, nucleotide-binding, leucine-rich repeats; CNL, coiled coil, nucleotide binding, leucine-rich repeats; RNL, *RESISTANCE TO POWDERY MILDEW 8* (*RPW8*) - nucleotide binding, leucine-rich repeats; NL, nucleotide-binding, leucine-rich repeats without a 5’ domain.

Supplemental Table S9

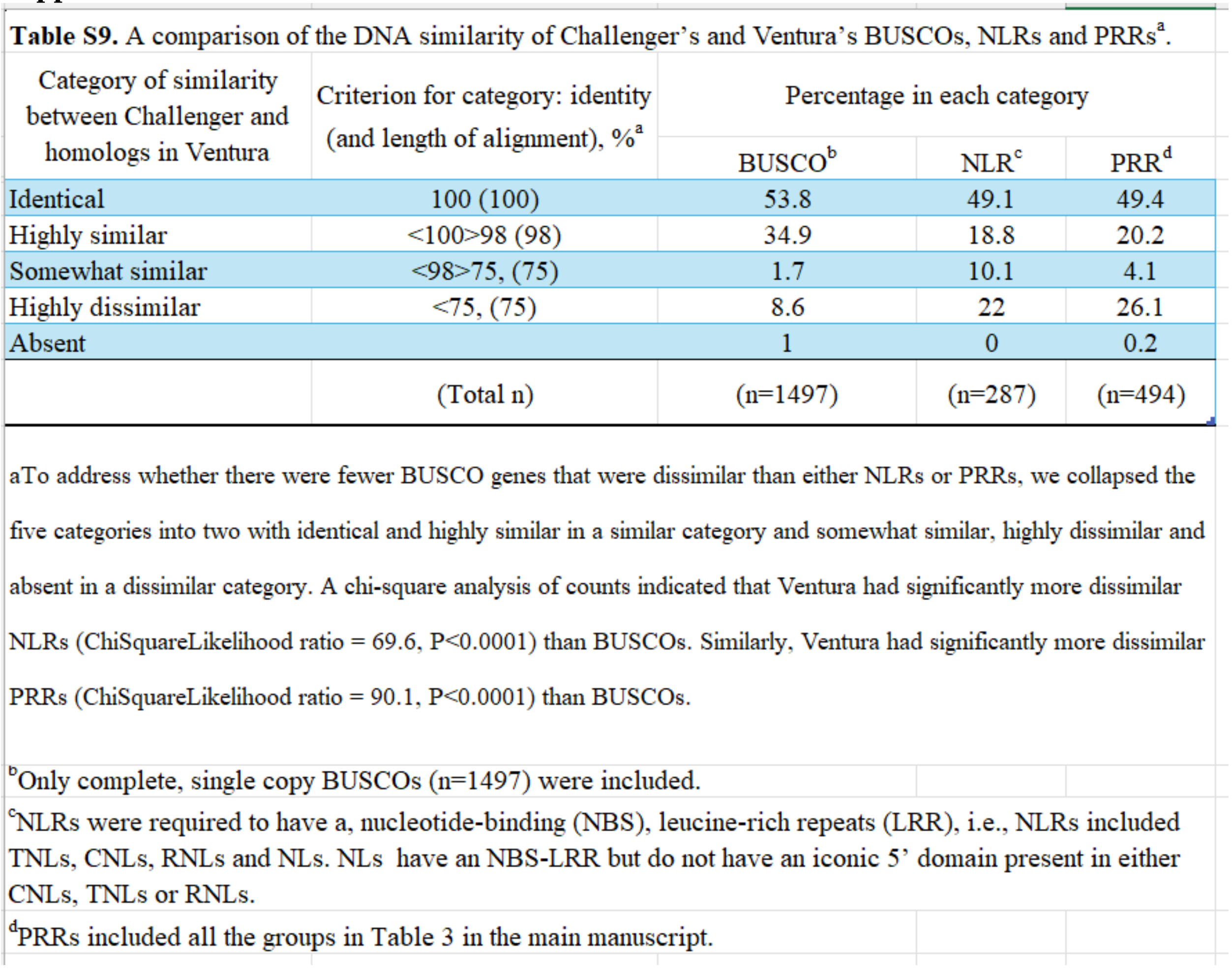


Table S9. A comparison of the DNA similarity of Challenger's and Ventura's BUSCOs, NLRs and PRRs[a].

Category of similarity between Challenger and homologs in Ventura	Criterion for category: identity (and length of alignment), %[a]	Percentage in each category		
		BUSCO[b]	NLR[c]	PRR[d]
Identical	100 (100)	53.8	49.1	49.4
Highly similar	<100>98 (98)	34.9	18.8	20.2
Somewhat similar	<98>75, (75)	1.7	10.1	4.1
Highly dissimilar	<75, (75)	8.6	22	26.1
Absent		1	0	0.2
	(Total n)	(n=1497)	(n=287)	(n=494)

aTo address whether there were fewer BUSCO genes that were dissimilar than either NLRs or PRRs, we collapsed the five categories into two with identical and highly similar in a similar category and somewhat similar, highly dissimilar and absent in a dissimilar category. A chi-square analysis of counts indicated that Ventura had significantly more dissimilar NLRs (ChiSquareLikelihood ratio = 69.6, P<0.0001) than BUSCOs. Similarly, Ventura had significantly more dissimilar PRRs (ChiSquareLikelihood ratio = 90.1, P<0.0001) than BUSCOs.

[b] Only complete, single copy BUSCOs (n=1497) were included.

[c] NLRs were required to have a, nucleotide-binding (NBS), leucine-rich repeats (LRR), i.e., NLRs included TNLs, CNLs, RNLs and NLs. NLs have an NBS-LRR but do not have an iconic 5' domain present in either CNLs, TNLs or RNLs.

[d] PRRs included all the groups in Table 3 in the main manuscript.

Supplemental Table S10

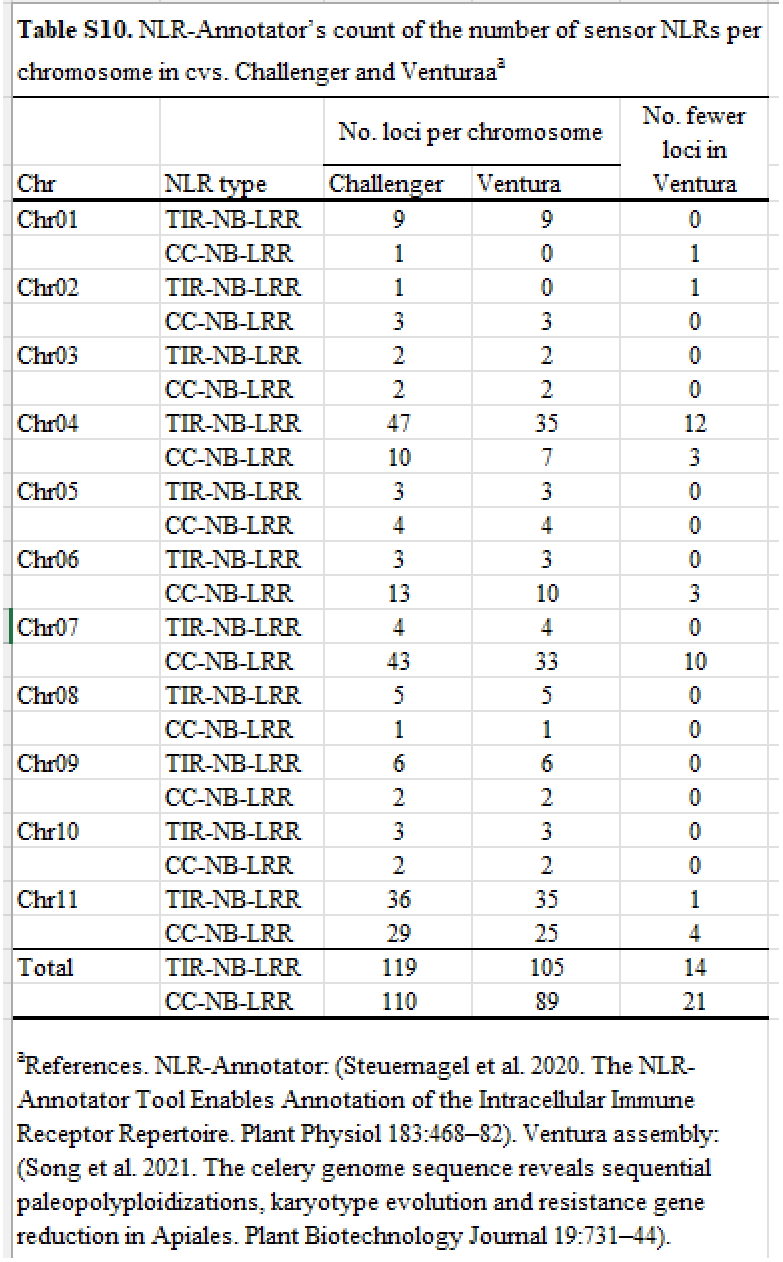


Table S10. NLR-Annotator's count of the number of sensor NLRs per chromosome in cvs. Challenger and Venturaa[a]

		No. loci per chromosome		No. fewer loci in
Chr	NLR type	Challenger	Ventura	Ventura
Chr01	TIR-NB-LRR	9	9	0
	CC-NB-LRR	1	0	1
Chr02	TIR-NB-LRR	1	0	1
	CC-NB-LRR	3	3	0
Chr03	TIR-NB-LRR	2	2	0
	CC-NB-LRR	2	2	0
Chr04	TIR-NB-LRR	47	35	12
	CC-NB-LRR	10	7	3
Chr05	TIR-NB-LRR	3	3	0
	CC-NB-LRR	4	4	0
Chr06	TIR-NB-LRR	3	3	0
	CC-NB-LRR	13	10	3
Chr07	TIR-NB-LRR	4	4	0
	CC-NB-LRR	43	33	10
Chr08	TIR-NB-LRR	5	5	0
	CC-NB-LRR	1	1	0
Chr09	TIR-NB-LRR	6	6	0
	CC-NB-LRR	2	2	0
Chr10	TIR-NB-LRR	3	3	0
	CC-NB-LRR	2	2	0
Chr11	TIR-NB-LRR	36	35	1
	CC-NB-LRR	29	25	4
Total	TIR-NB-LRR	119	105	14
	CC-NB-LRR	110	89	21

[a] References. NLR-Annotator: (Steuernagel et al. 2020. The NLR-Annotator Tool Enables Annotation of the Intracellular Immune Receptor Repertoire. Plant Physiol 183:468–82). Ventura assembly: (Song et al. 2021. The celery genome sequence reveals sequential paleopolyploidizations, karyotype evolution and resistance gene reduction in Apiales. Plant Biotechnology Journal 19:731–44).

The levels of expression of the 243 NLRs varied (Main Figure 3b), Because documented NLR R genes in various pathosystems are typically comparatively highly expressed (Brabham *et al.*, 2024), we examined the NLRs that were in the upper quartile in expression in either the mock-infested or infested with *Foa* race 2. We then selected the highly expressed NLRs that had homologs in Ventura that were dissimilar from those in Challenger; 17 NLRs in the upper quartile of expression had less than 75% identity in Ventura, and 4 had between 75% and 98% identity . The 21 selected

NLRs include 9 CNLs, 5 TNLs and 7 NLs. Sixteen of these NLRs are in three locations: six of eight selected NLRs from chromosome 4 could be introgressed from a recombinant from the terminus of chromosome 4, 9.15 Mb from NLR 80 through 27; all six selected NLRs on chromosome 6 could be introgressed in a 24 Mb region (Chr6 NLRs 14-22); and all four selected NLRs on chromosome 11 could be introgressed in a 10.3 Mb region (Chr 11 NLRs 17-65). The five additional highly expressed NLRs are located at the other terminus of chromosome 4 and on chromosome 7. These NLRs were considered to be the most likely to be associated with *Foa* race 2 resistance and the most easily tested because they can be readily introgressed into a susceptible variety.

Because NLRs are often constitutively expressed (Von Dahlen *et al*., 2023) and we had no evidence of either up- or downregulated transcriptional regulation of Challenger's NLRs associated with either the pathogen treatments (mock, *Foa* race 2, or *Foa* race 4), days post-infestation (dpi) (7, 14, and in some cases 21 dpi) or the pathogen–dpi interaction, we mapped reads from a previous trial (Henry *et al.*, 2020) in Tall Utah 52-70R Improved, which is susceptible to *Foa* race 2 and race 4, is an earlier variety than Ventura, and is presumably within Ventura's clade (Quiros, 2016). The Tall Utah trial was similar to the Challenger trial but differed in two aspects: 1) instead of dipping the crowns and roots in the inoculum, the plants were transplanted into infested soil, and the onset of disease was later than that in the Challenger trial, and 2) the crowns were only harvested at 21 dpi. Because the two trials were independent, we cannot compare the TagSeq DESeq2-normalized counts between the two trials. However, as an indication of presence/absence expression in the Ventura lineage, we used the Tall Utah dataset to look for those homologs that differed qualitatively, i.e., expressed in Challenger and either not expressed or negligibly expressed in Tall Utah. Overall, log (DESeq2-normalized expression +1) in Challenger and Tall Utah in the 21 dpi mock treatments were highly significantly correlated ($r=0.87$, $P<0.0001$). However, among the 243 NLRs that were expressed in Challenger crowns, three were selected as potential candidates for *Foa* race 2 resistance (Main Figure 2c). All three NLRs are in the upper quartile of expression in Challenger and are either not expressed or negibily expressed in Tall Utah. Chromosome 4 NLR 69 (AgCh.04.g150590) is a 5121 bp TNL in a cluster; its Ventura homolog has a SNP in the start codon and then a 5' truncation that results in a frame shift mutation that results in many stop codons. In addition, there is a 3' truncation in Ventura in the final exon that deletes a C-JID domain. Chromosome 4 NLR 56 (AgCh.04.g150320) is a 5906 bp TNL; its homolog in Ventura has 5' and 3' truncations. While there is one alternative start codon, the 3' truncation reduces the number of LRRs from 12 in Challenger to three. Chromosome 6 NLR 18 (AgCh.06.g193140) is a 3019 bp NL in a cluster. All three NLRs are located within the higher recombining regions and could be introgressed into a cultivar that is *Foa* race 2-susceptible in order to determine if these particular NLRs or clusters confer *Foa* race 2 resistance. To summarize, although we can't prove that at least one of these three NLR candidates for *Foa* race 2 resistance is actually the R gene that confers Foa race 2 resistance, it wouldn't be much work to test the hypothesis.

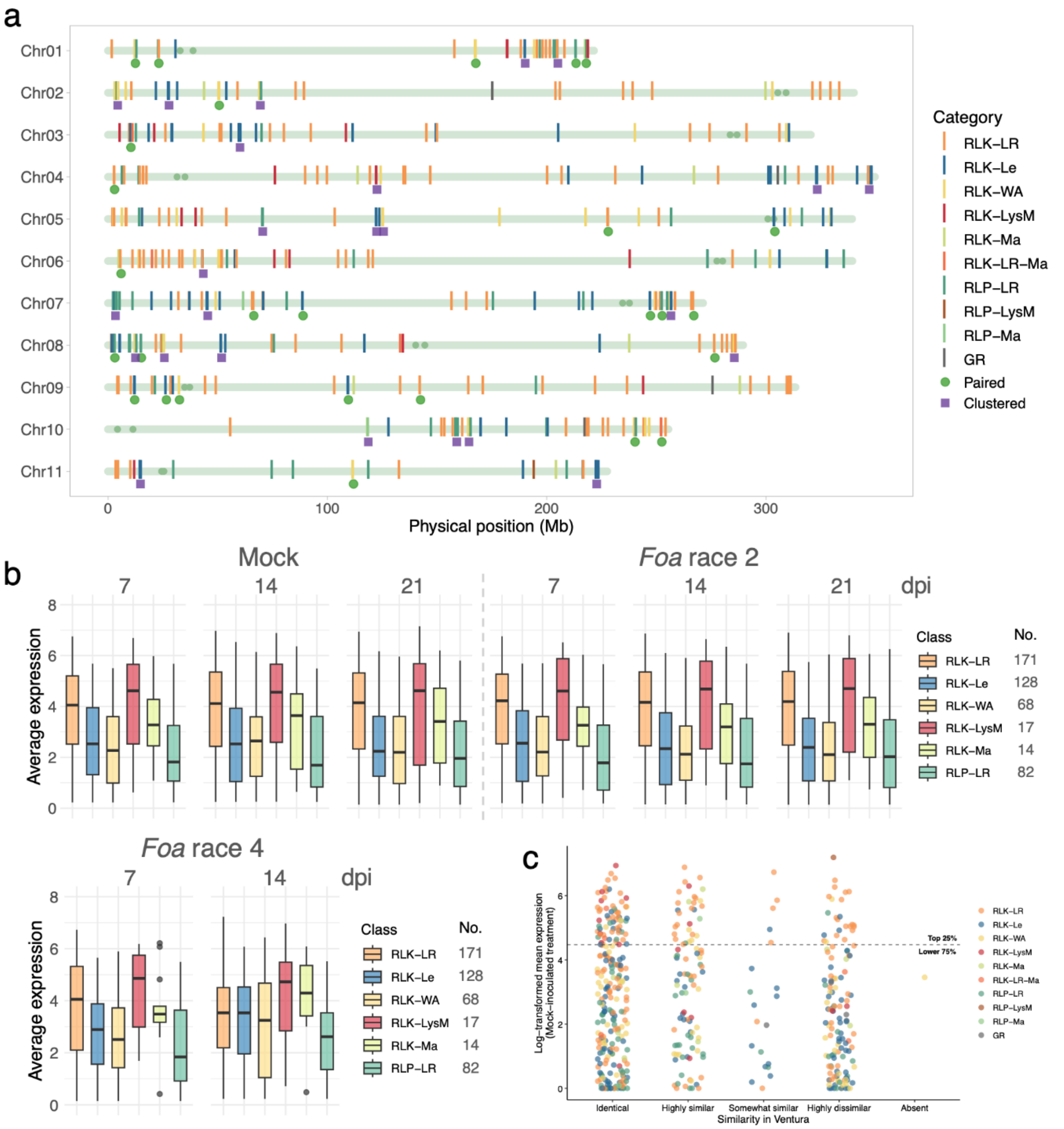
Consequently, we propose a straightforward strategy for determining if introgression of some of Challenger's highly clustered NLRs will improve resistance to *Foa* race 2. More specifically, any

Foa race 2-susceptible celery cultivar could be crossed with Challenger. We note that using the susceptible variety as the female parent has the advantage that any undesired selfs instead of F1s could be removed by screening the F1 for race 2-resistance, preferably in carefully controlled conditions for the most accurate phenotyping. Then, a population of F1s could be selfed, and, if informative PCR primers were selected, the F1S1 seedlings could be screened by PCR for the NLR zones of interest and tested for race 2-resistance.

Together with NLRs, the PRRs are a critical group of genes that encode for Resistance (R) genes in many molecularly characterized plant-pathogen interactions, including in plant-*F. oxysporum* interactions. Similar to the situation with NLRs, although we didn't have a biological experiment in which we could identify whether any particular PRR was a R gene to *Foa* race 2, we wanted a catalog of the PRRs in Challenger, at the least because it is the *Foa* race 4-susceptible parent to F1S3. PRRs are part of pattern triggered immunity (PTI), and extracellularly detect pathogen-associated molecular patterns (PAMPs), microbe-associated molecular patterns (MAMPs) and damage-associated molecular patterns (DAMPs) (Zhang *et al.*, 2024). There are two main sub-groups in the PRRs: the receptor-like kinases (RLKs), which we divided into six subgroups on the basis of their extracellular domains; and the receptor-like proteins (RLPs), which we divided into 3 subgroups (Main Table 3). Although PRRs (Main Figure 3a) are far less clustered than NLRs (Main Figure 2a) and are less concentrated in the higher recombination region than in the lower recombination region compared to the NLRs, they are significantly more represented in the higher recombining region than either total genes or BUSCO genes. (Supplemental Table S6).

In the three treatments (mock-infested, infested with either *Fo*a race 2 or *Foa* race 4), Challenger expressed 88% and 77% of their cell surface-RLKs and RLPs, respectively, in their crowns: 155 RLK-LR, 107 RLK-Le, 59 RLK-WA, 16 RLK-LysM, 13 RLK-Ma, and 5 RLK-LR-Ma, with a total of 355 cell surface receptor-like kinases and 66 receptor-like proteins, 63 of which are RLP-LRs. Main Figure 3b shows the variation in the levels of expression of log DESeq2-normalized counts of the expressing RLKs and RLPs that have more than 10 members in the subgroup. The point is that many of the RLKs and RLPs are quite highly expressed, which suggests that they are important in Challenger. We used BLASTn to identify homologous PRRs in the Ventura genome. One RLK-WA (AgCh.05.g178690) is apparently absent in Ventura, and 31% were classified as highly dissimilar (<75% identity in 75% of the length of the Challenger model) (Main Table 3). Among the other subgroups with > 20 members, the Ventura homologs were highly dissimilar in 25% of the RLK-LR, 33% of the RLK-Le and 17% of the RLK-LR (Main Figure 3c). Similar to the NLRs, there were significantly more PRRs ($P_{\text{chi-square}}<0.0001$) in Ventura that were dissimilar to Challenger's gene models than to the BUSCO genes (Supplemental Table S9).

Results using the GBS protocol to track diversity within *A. graveolens* accessions were mostly shown in last year's report. The protocol for celery is as follows. The fourth leaves of the plants were lyophilized, ground in liquid N_2 in a mortar and pestle and purified via the Macherey-Nagel Nucleospin Plant II Kit following the manufacturer's protocol, except that the RNase step was extended to 30 min. DNA was digested with the blunt-end cutting restriction enzyme MlyI using the Hill et al. GBS protocol (Hill *et al.*, 2023). Sequencing libraries were constructed with an average size of 300–350 bp (Monson-Miller *et al.*, 2012). The quality assessment of the GBS data was performed via FastQC v0.11.9 (Andrews, 2010). After the raw reads were polished via Trimmomatic v0.39 (Bolger *et al.*, 2014), the trimmed reads were mapped to the Challenger genome via BWA v0.7.17 (Li, 2013). Variant calling was conducted via the MultisampleVariantsDetector module from NGSEP 4 (Tello *et al.*, 2023) with default parameters. The resulting VCF file was filtered to retain the biallelic variants called from all 11 samples with depths greater than one.



Main Fig. 3. Potential pattern recognition receptors (PRRs) in Challenger. **a** Chromosomal locations of 494 gene models of PRRs. There are six subgoups with an intracellular receptor-like kinase (RLK) with the following extracellular binding domains: RLK-LR, leucine-rich repeats; RLK-Le, a lectin domain, typically for binding mannose; RLK-WA, an intracellular wall-associated kinase and typically a galacturonan binding domain; RLK-LysM, with a chitin-binding, LysM domain; and RLK-Ma, with a malectin-binding domain. There are three groups of intracellular receptor-like proteins (RLPs) with the following extracellular binding domains: RLP-LR, with leucine-rich repeats; RLP-LysM, with a LysM domain; and RLP-Ma, with a malectin domain. GR, glutamate-gated receptor. PRRs are designated pairs when there are two adjacent members in the same subgroup or clusters when there are three or more consecutive members in the same subgroup. Within the chromosome line, small circles represent the start and end of the centromeric region. **b-c**. The plants were mock-infested or infested with either *Foa* race 2 or *Foa* race 4 and then harvested at the indicated time points. **b** Box plots of log DESeq2-normalized counts of 480 PRRs in Challenger crowns. The whiskers are 1.5 times the interquartile range (IQR) from the lower quartile to the upper quartile. **c** Distributions of DESeq2-normalized counts of 494 PRRs in Challenger crowns: Categories are based on the similarity of Ventura's orthologous DNA to the Challenger's DNA sequence: identical, identical in 100% of the length; highly similar, identical in <100≥98% in 98% of the length: somewhat similar, identical in <98≥75% in 75% of the length; and highly dissimilar, <75% identical in 75% of the length.

Main Table 3. Based on cell-surface Pattern Recognition Receptors in Challenger, the similarity of homologs in cv. Ventura†

Category of similarity between Challenger PRRs and homologs in Ventura	Criterion for category: identity (and length of alignment), %^{a}	No. in each category (Expressed in Challenger, %)									
		RLK-LR‡	RLK-Le§	RLK-WA¶	RLK-LysM^{e}	RLK-Ma^{f}	RLK-LR-Ma^{g}	RLP-LR^{h}	RLP-LysM^{i}	RLP-Ma^{j}	GR^{k}
Identical	100 (100%)	89 (93)	60 (90)	29 (97)	9 (89)	6 (100)	3 (100)	45 (73)	-	1(0)	-
Highly similar	<100≥98 (98%)	33 (88)	16 (94)	17 (100)	6 (100)	5 (100)	-	20 (85)	-	2 (100)	-
Somewhat similar	<98≥75 (75%)	6 (83)	10 (80)	-	-	-	-	3 (100)	-	-	1 (100)
Highly dissimilar	<75 (75%)	43 (98)	42 (86)	21 (71)	2 (100)	3 (100)	2 (100)	14 93)	1 (100)	-	4 (75)
Absent^{l}		-	-	1 (100)	-	-	-	-	-	-	-
Total		171 (93)	128 (88)	68 (90)	17 (94)	14 (100)	5 (100)	82(80)	1(100)	3(67)	5(80)

†PRRs, pattern recognition receptors, are proteins on the plant cell surface that detect conserved patterns associated with either microbes or with host cell damage. All the entries have either a transmembrane or a GPI anchor domain and a designated cell membrane localization by DeepLoc 2.1.

‡RLK-LRs are iconic PRRs with an intracellular receptor-like kinase and extracellular leucine-rich repeat domains. Typically, Challenger's RLKs have a Pkinase_Tyr catalytic domain, and the LRR has LRRNT_2, LRR_1, LRR_6, and LRR_8 Pfam domains.

§RLK-Le contains an intracellular kinase domain and an extracellular lectin-like receptor, which binds a specific carbohydrate. Fungal extracellular and wall proteins are typically mannosylated, and Challenger RLK-Lec typically have an extracellular bulb-type lectin (B_lectin) domain, which is also called a G-type lectin S-receptor-like domain, which binds mannosyl residues. Typically, Challenger's RLK-Le have a Pkinase_Tyr catalytic domain, a DUF3403 domain and a PAN-2 Pfam domain. Approximately 8% of Challenger's lectins have an L-type legB domain.

¶RLK-WA, wall-associated kinase containing an intracellular kinase domain and an extracellular galacturonan-binding domain. Challenger RLK-WA typically have a Pkinase_Tyr and a GUB_WAK binding domain. Many also have an EGF_CA-binding domain.

[e] RLK-LysM have an intracellular kinase and an extracellular LysM domain, which bind chitin, a constituent of fungal walls. Some LysM domains also bind peptidoglycan oligosaccharides (Willmann *et al.*, 2011)

[f] RLK-Ma contain an intracellular kinase domain (Pkinase_Tyr) and an extracellular malectin-like domain. Malectin can bind to multiple cysteine-rich peptides named rapid alkalinization factors (Franck *et al.*, 2018).

[g] RLK-LR-Ma contain an intracellular kinase domain and extracellular leucine-rich repeats and malectin domains.

[h] RLP-LR, an iconic PRR with extracellular leucine-rich repeats. Challenger RLP-LR typically have LRRNT_2, LRR_1, LRR_6, and LRR_8 Pfam domains.

[i] RLP-LysM have an extracellular LysM domain.

[j] RLP-Ma have an extracellular malectin-like domain.

[k] GR, glutamate-gated receptor. The genes have an ANF_(peptide) receptor, a Lig_chan, and an SBP_bac_3 domain. It is unknown whether GRs are legitimate PRRs.

[l] Absent, no BLAST hits

Objective 2. To produce a high-quality assembly and annotation of a celery breeding line (F1S3 76-8-36-124) which is resistant to *Fusarium oxysporum* f. sp. *apii* (*Foa*) races 4 and 2. All the specific sub-objectives are in progress. We intend to submit a publication in November 2025:

Objective 2a. To assemble a high-quality chromosome-level genome of the race 4-resistant celery breeding line (F1S3 76-8-36-124), which is fixed (=homozygous) for resistance to *Fusarium oxysporum* f. sp. *apii* race 4;

In our last year's report, submitted Mar. 2024, we reported improvements of "manual" evaluation of certain areas in which depth coverage or other evidence indicated that there were mis-assembly and mis-scaffolding. Here, we show a comparison of the F1S3 in comparison to Challenger. The finer scale results will be useful in completion of Objective 2d below.

In the early steps in gene annotation, we bioinformatically detected approximately 127,000 potential genes, which is approximately three times more genes than are present. Currently, we are removing all of the "potential genes" that overlap with repetitive elements, i.e., those that are not actual genes. We are currently using the datasets in Objectives 2b and 2c to identify the supported "gene models."

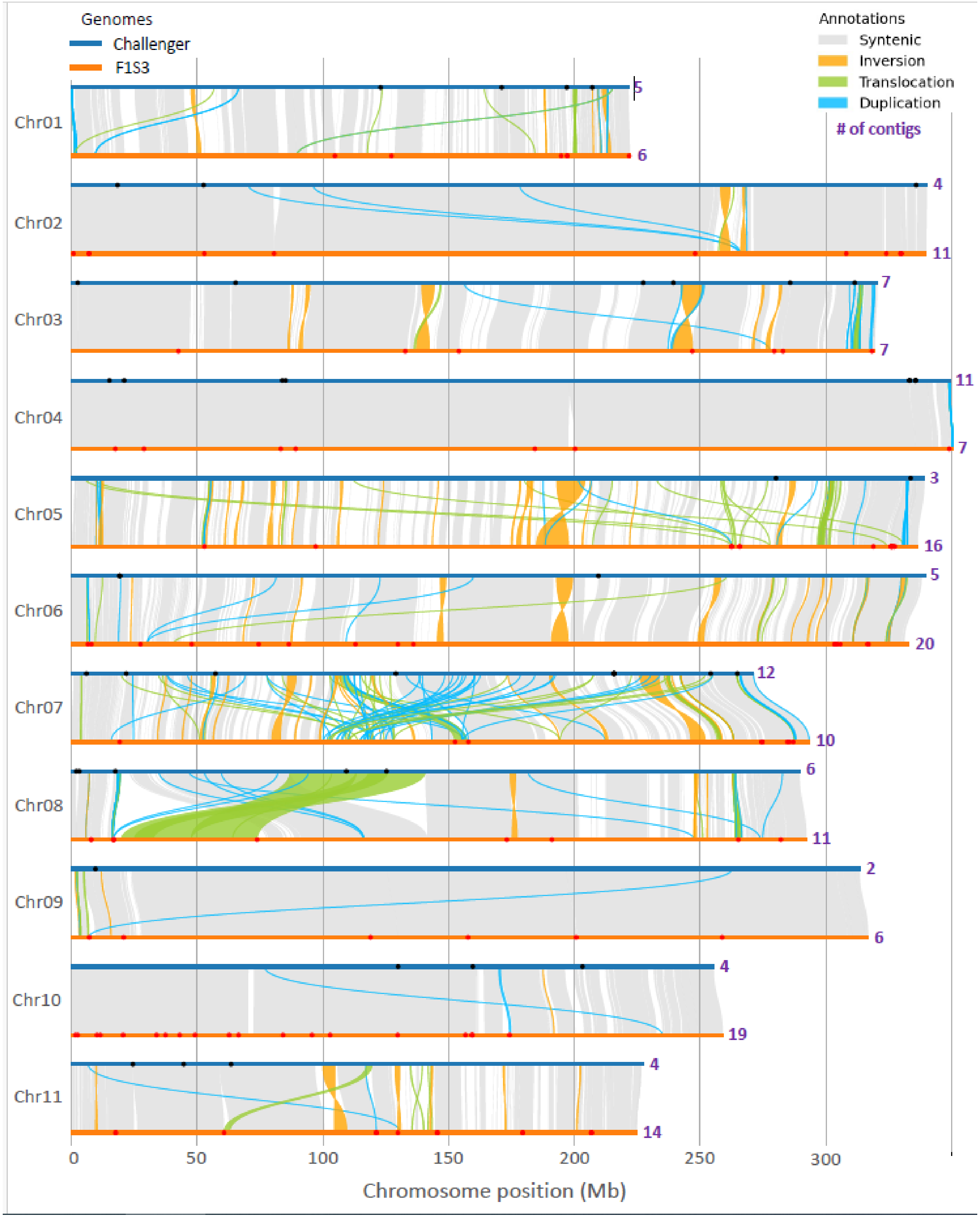

Genomes
Challenger
F1S3
Annotations
Syntenic
Inversion
Translocation
Duplication
# of contigs
Chr01
5
6
Chr02
4
11
Chr03
7
7
Chr04
11
7
Chr05
3
16
Chr06
5
20
Chr07
12
10
Chr08
6
11
Chr09
2
6
Chr10
4
19
Chr11
4
14
0
50
100
150
200
250
300
Chromosome position (Mb)

Fig. 4. A comparison of the Challenger (upper line) and F1S3 (lower line) genomes by chromosome (Chr). Syntenic (highly similar) regions, which are connected by gray lines, had a minimum of 90% identity with a minimum alignment length of 100 base pairs (bp). The number of contigs (segments of DNA sequence/chromosome) is indicated at the end of each chromosome.

Objective 2b. To collect and use Iso-Seq data (full length transcripts) to annotate the genes in F1S3 76-8-36-124 crowns and roots under race 4 disease pressure;

We have generated 18,770 Iso-Seq (presumably full-length) gene models for F1S3, derived from pooled root samples collected during experiments conducted in the winter 2023 and summer 2024. Our current annotation demonstrates remarkable completeness with 98.1% BUSCO score with only 11 missing BUSCOs **(Table 4)**. To discover alternative isoform information and improve gene model structures, we are refining the current gene models using the full-length transcript evidence from Iso-Seq data.

Table 4.

Table 4. BUSCO scores for the current F1S3 genome assembly in contig-level, chromosome-level, and annotation-level

	Contigs		**Chromosomes**		**Annotation**	
	#BUSCOS	%BUSCOs	#BUSCOS	%BUSCOs	#BUSCOS	%BUSCOs
Complete	1599	99.1	1599	99.1	1590	98.5
Complete, single-copy	1498	92.8	1507	93.4	1503	93.1
Complete, duplicated	101	6.3	92	5.7	87	5.4
Fragmented	13	0.8	13	0.8	11	0.7
Missing	2	0.1	2	0.1	13	0.8
Total	1614	100	1614	100	1614	100

Objective 2c. To collect and use Tag-Seq (=RNA-Seq) data of F1S3 76-8-36-124 race 4-resistant vs. Challenger (race 4-susceptible) to i) identify candidate resistance and susceptibility genes in the breeding line and in celery, respectively, ii) to identify candidate "effector" genes in *Foa* race 4 that affect the outcome of the host-pathogen interaction and iii) to help understand the "downstream" pathways that result in either resistance or susceptibility;

This objective is in progress. We have generated all the 3' Tag-Seq data, which exceeds the amount of our previous replicated Challenger dataset (which had crowns from just Challenger uninfested, or infested with either *Foa* race 2 or *Foa* race 4) at several time points. The current replicated trial has roots from F1S3 and Challenger in either uninfested or infested with *Foa* race 4 at several time points. The data will be used, along with Objective 2b for genome annotation, for assistance in identifying resistance gene candidates in Objective 2d for resistance in F1S3 to Foa race 4 and, to identify pathogen effectors and downstream genes involved in resistance in F1S3 and susceptibility in Challenger this Objective 2cii and 2ciii to *Foa* race 4. We hope that we will be able to develop a 3rd manuscript from the information in Objective 2cii and 2ciii.

Objective 2d. To use all the available datasets (including the Illumina sequence from bulk segregant analysis of race 4-resistant vs. susceptible populations, the F1S3 assembly from Objectivs 2a and 2b, and the Challenger genome assembly) to identify candidate genes that confer resistance to *Foa* race 4 in celery.

This is in progress! As mentioned above, we intend to submit a manuscript on this Nov. 2025.

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California Celery Research Advisory Board Report

Project title: Year 3: Celery breeding for resistance to *Fusarium oxysporum* f. sp. *apii* race 2 and race 4

Period of Funding: January 1, 2024 to September 30, 2024

Researchers:

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For questions or concerns, please contact Renee Eriksen at renee.eriksen@usda.gov

Specific Objectives

1. *Continue development of UCD resistant germplasm lines*
2. *Development of new resistant germplasm lines*
3. *Evaluate these germplasm lines and additional material in the field*
4. *Evaluate performance of a susceptible cultivar with additional micronutrient amendments*
5. *Outreach*

Objective 1: Continue development of UCD resistant germplasm lines.

In the fall of 2023, plants from the UCD germplasm lines 76-12-1, 77-27-16, 76-8-36-124, 76-8-36-133, 76-8-36-139 were included in the *Fusarium oxysporum* f. sp. *apii* race 4 (FOA4) field trial in Camarillo. The germplasm line UCD 76-8-36-124 was released by UCD and Dr Epstein in 2022 and has fixed resistance to FOA4. The primary factor influencing the decision to include the other germplasm lines in the trial was the availability of seed; we had a lot of seed available for these lines. We made selections for improved agronomic traits from these lines, emphasizing thick, compact petioles. No plants from the lines 76-12-1 or 77-27-16 line were selected due to high disease incidence and poor celery morphology. Selected plants from UCD 76-8-36-124, 76-8-36-133, and 76-8-36-139 were removed from the field, and transported to the Sam Farr Crop Improvement and Protection Research Unit in Salinas, California. The plants were vernalized at 4ºC, and moved to greenhouse isolator units for selfing and back-crosses during the summer of 2024. One plant of each 76-8-36-124, 76-8-36-133, 76-8-36-139 were back-crossed to cv. Challenger to attempt to improve the celery morphology, and future work will need to re-select disease resistance. Seed from these lines will be grown in the 2025 field trials and in Salinas, and further selected for improved celery morphology.

Objective 2: Development of new resistant germplasm lines

We made crosses with cv Challenger (containing genetic resistance to FOA2) and an OP heirloom variety called French Dinant with strong field tolerance to FOA4 to develop additional germplasm lines with stacked resistance to FOA2 and FOA4. The development of additional germplasm lines will increase the available germplasm with FOA4 resistance. Seed from these crosses will be field tested in the 2025 field trials to screen for disease resistance.

In 2024, we optimized procedures for inoculating celery with FOA4 in the greenhouse. We tested three media (potting soil, sand, 1:1 mix of potting soil and sand), four inoculation methods (FOA4 infested millet grains, a FOA4 liquid media poured over the roots, a FOA4 liquid media root soak, and a FOA4 liquid media root soak with purposefully damaged roots), and four age classes of plants (40 day old plants, 65 day old plants, 90 day old plants, and 150 day old plants). We ran four experiments each for eight weeks, each containing 240 plants exposed to various combinations of the conditions described above. We concluded that 65 day old plants in potting soil with a FOA4 liquid media poured over the roots produces the strongest and most consistent symptoms of disease. Additionally, we tested plants under four different temperature treatments: 64ºF , 70ºF, 75ºF, and 80ºF. As the Board is aware, soil temperatures over 70ºF produced rapid decline and plant death. This work allowed us to optimize our inoculation methods, and a detailed description of our methods will be made available to the celery breeding and scientific community. Interested parties can contact renee.eriksen@usda.gov for our protocols. We will begin experiments in 2025 to evaluate over 100 germplasm lines from various germplasm banks, including the USDA-GRIN-Global collection, for FOA4 resistance. Resistance information will be shared with the celery breeding community so they may use that information to develop new germplasm lines. Resistance information will also be used to develop new pre-breeding germplasm lines with FOA4 resistance at the USDA celery breeding program.

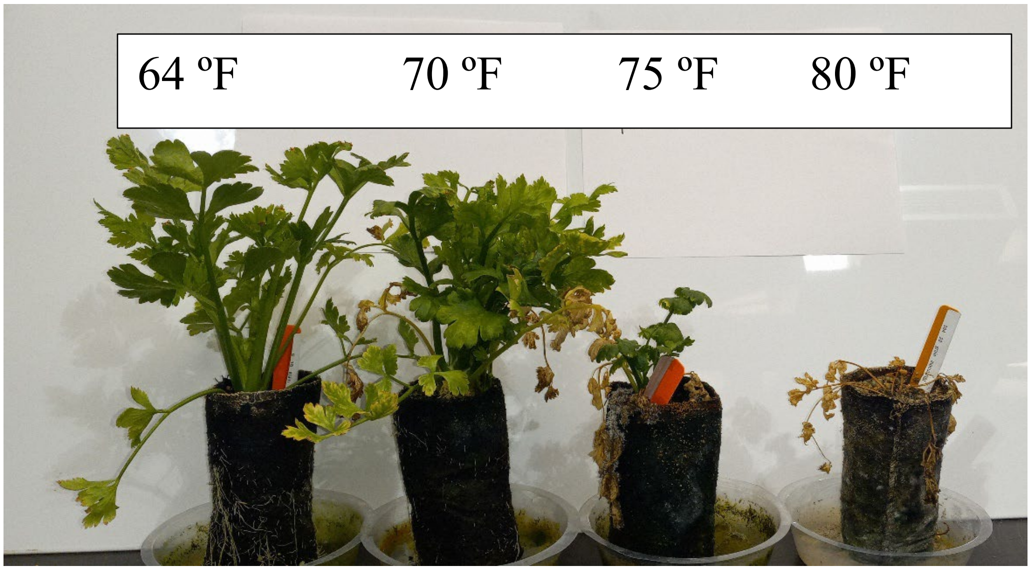

Fig. 1: Cv. Sonora plants inoculated with millet grains that were infested with FOA4 and grown in growth chambers at 64ºF, 70ºF, 75ºF, and 80ºF. The plants were exposed to FOA4 for eight weeks. All plants received the same treatment except temperature, which resulted in significantly more symptoms and plant death at temperatures above 70 ºF.

Objective 3: Evaluate germplasm and additional material in the field

The trials were conducted during the following dates:

TRIAL	TRANSPLANT DATE	HARVEST DATE
SANTA MARIA FOA2	June 11, 2024	September 18-19, 2024
CAMARILLO FOA4	August 13, 2024	December 5-6, 2024

Table 1: Disease evaluation ratings

SCORE	CRITERIA
0	No browning
1	Browning visible, particularly in feeder roots
2	Browning visible, particularly in primary roots
3	Browning visible, particularly around the crown
4	Browning in and/or around the crown
5	Extensive browning in crown, decay in crown tissue
6	Plant dead or missing due to death

Table 2: Agronomic trait ratings:

TRAIT	UNITS	METHOD
PETIOLE LENGTH	inches	Petioles were cut at approximately 1-2 inches above the average first petiole node and measured. This trait captured stunting due to disease as well as general agronomic characteristics, and was one of two quantitative measurements.
PLANT WEIGHT	lbs	Ten trimmed plants were weighed to the nearest kg and an average plant weight was calculated. Units were converted to pounds (lbs) for this report. As with petiole length, this trait captured stunting due to disease as well as general agronomic characteristics and was one of two quantitative measurements.
GREENNESS	Score 1-3	This trait describes the color of the stalk. A score of 1 was particularly light or yellow, and a score of 3 was particularly dark. This trait was highly dependent on the individual conducting the scoring and on the light during which the evaluation was made. It could also be highly dependent on nitrogen fertilization, though we assume fertility was consistent among blocks.
PETIOLE CRACKING	Score 1-3	A score of 1 is no cracking at the base, a score of 2 is minor cracking at the base, and a score of 3 is severe cracking at the base.
BOLTING	Score 1-3	A score of 1 indicates no bolting; 2 indicates the beginning of a bolt stalk, 3 indicates active flowering.
PITHINESS	Score 1-3	This trait describes hollow, pithy stems which are common in wild celery or lovage, but a defect in stalk celery. A score of 1 was no observed pithiness at any cut. A score of 2 was a small amount of pithiness in less than half of the stalks. A score of 3 was pithiness in >50% of stalks.
RIBBINESS	Score 1-3	A score of 1 was particularly smooth, and a score of 3 was particularly ribby or rough such that the ribs cast shadows. A score of 2 was intermediate. Ribbiness appeared to increase under disease pressure.
SUCKERING	Score 1-3	A score of 1 had no suckering, 2 had some suckering, and 3 had consistent suckers, similar to wild celery. Suckering seemed to increase with race 4 pressure in some cultivars.

Santa Maria FOA2 trial

Methods: A total of 28 entries were received for the FOA2 trial. Seeds were submitted by Bejo Seeds, Rijk Zwaan, Syngenta, and Tozer Seeds. There were 12 beds, with two beds filled with a border row of a field variety on either side. Beds were organized into four replicate blocks (Reps). Within each Rep, there were eight beds divided into five 20-foot plots. Germplasm entries were randomized within each Rep independently (see map in section Supplemental Materials). The susceptible control was cv. Tall Utah 52-70, acquired from Ferry Morse Seed Company. The resistant control was cv. Challenger derived from the UC Davis breeding program (Orton et al. 1984) by Pybus Seeds. Harvest and disease evaluation occurred on September 18+19, 2024, after approximately 100 days. Twenty plants were removed from the center of each plot; ten were evaluated for root and crown discoloration indicative of disease, the other ten were evaluated for agronomic traits. Disease evaluations were conducted by one person per Rep to confine the assessors' scoring approaches to respective blocks.

Results: Disease scores ranged from 0-5. Cultivar names below are reported alphabetically when scores are equal. Names beginning with BJ are submissions from Bejo Seeds, RZ are submissions from Rijk Zwaan, and TZ are submissions from Tozer Seeds.

The germplasm entries with the lowest average disease score (a low disease score = a healthy plant) were RZ2003 (average score = 0.05) and TZ6 (average score=0.05), following by BJH10044 (average score 0.08). RZ2003 was taller, at 15.9 inches, with a total average plant weight of 1.6 lbs. It was moderately ribby (score 2.1 out of 3) with some pith detected (score 1.9 out of 3) and some suckering, score 2.0 out of 3). TZ6 was slightly smaller with average values of 13.2 inches tall and 1.4 lbs per trimmed plant. It had slightly more prominent ribs (score 2.2 out of 3), but more suckering (score 2.8 out of 3). BJH10044 was slightly smaller with an average height of 11.2 inches, 1.1 lbs. It was slightly less ribby (score 2.0 out of 3), with similar pithiness (score 1.6 out of 3), and less suckering than TZ1 (score 2.1 out of 3).

The germplasm entries with the highest disease scores were Tall Utah (susceptible control, average score = 3.53), Sonora (average score 3.50), and Mission (average score 3.48). The resistant control cv. Challenger derived from FOA2 resistant UC1 germplasm had an average score of 0.55, which is a higher average disease score than 14 of the 28 germplasm entries. We observed stunting in susceptible Tall Utah, but also in Sonora, Mission, and Conquistador. We also observed low average plant weight in Tall Utah, Sonora, Mission, Conquistador, RZ2001, and Command.

Conclusions: Germplasm entries such as RZ2003, TZ6, and BJH10044, BJH10031, BJH10043, and TZ1 had good field resistance to FOA2 in this trial.

Table 3: Cultivar entries in the **Santa Maria FOA2 trial 2024**. Cultivars are arranged alphabetically by breeding company or source

Cultivar	Source	
BJH10029	Bejo Seeds	
BJH10031	Bejo Seeds	
BJH10033	Bejo Seeds	
BJH10034	Bejo Seeds	
BJH10036/Bolero	Bejo Seeds	
BJH10039/Fandango	Bejo Seeds	
BJH10042	Bejo Seeds	
BJH10043/Merengo	Bejo Seeds	
BJH10044	Bejo Seeds	
BJH10045/Waltz	Bejo Seeds	
Tall Utah	Ferry Morse	Susceptible Control
RZ2001	Rijk Zwaan	
RZ2002	Rijk Zwaan	
RZ2003	Rijk Zwaan	
Celx767	Syngenta Seeds	
Challenger	Syngenta Seeds	Resistant Control
Command	Syngenta Seeds	
Conquistador	Syngenta Seeds	
Mission	Syngenta Seeds	
Sonora	Syngenta Seeds	
Stix	Syngenta Seeds	
TZ1	Tozer Seeds	
TZ2	Tozer Seeds	
TZ3	Tozer Seeds	
TZ4	Tozer Seeds	
TZ5	Tozer Seeds	
TZ6	Tozer Seeds	
TZ7	Tozer Seeds	

Table 4: Average disease rating of cultivar entries in the **Santa Maria FOA2 trial 2024**. Arranged from low to high rating. Low ratings indicate low disease and a healthy plant, and high ratings indicate more disease.

Disease Scores Lower than Challenger	
Cultivar	**Average Disease Score**
RZ2003	0.05
TZ6	0.05
BJH10044	0.08
BJH10031	0.10
BJH10043	0.10
TZ1	0.10
TZ3	0.15
BJH10039	0.23
Celx767	0.28
BJH10042	0.33
BJH10036	0.38
Stix	0.40
BJH10034	0.53
BJH10045	0.53
Challenger	0.55

Disease Scores Higher than Challenger	
Cultivar	**Average Disease Score**
RZ2002	0.60
TZ5	0.65
TZ2	0.70
BJH10033	0.83
TZ7	0.85
TZ4	0.90
BJH10029	0.95
Command	2.15
RZ2001	2.35
Conquistador	3.30
Mission	3.48
Sonora	3.50
Tall Utah	3.53

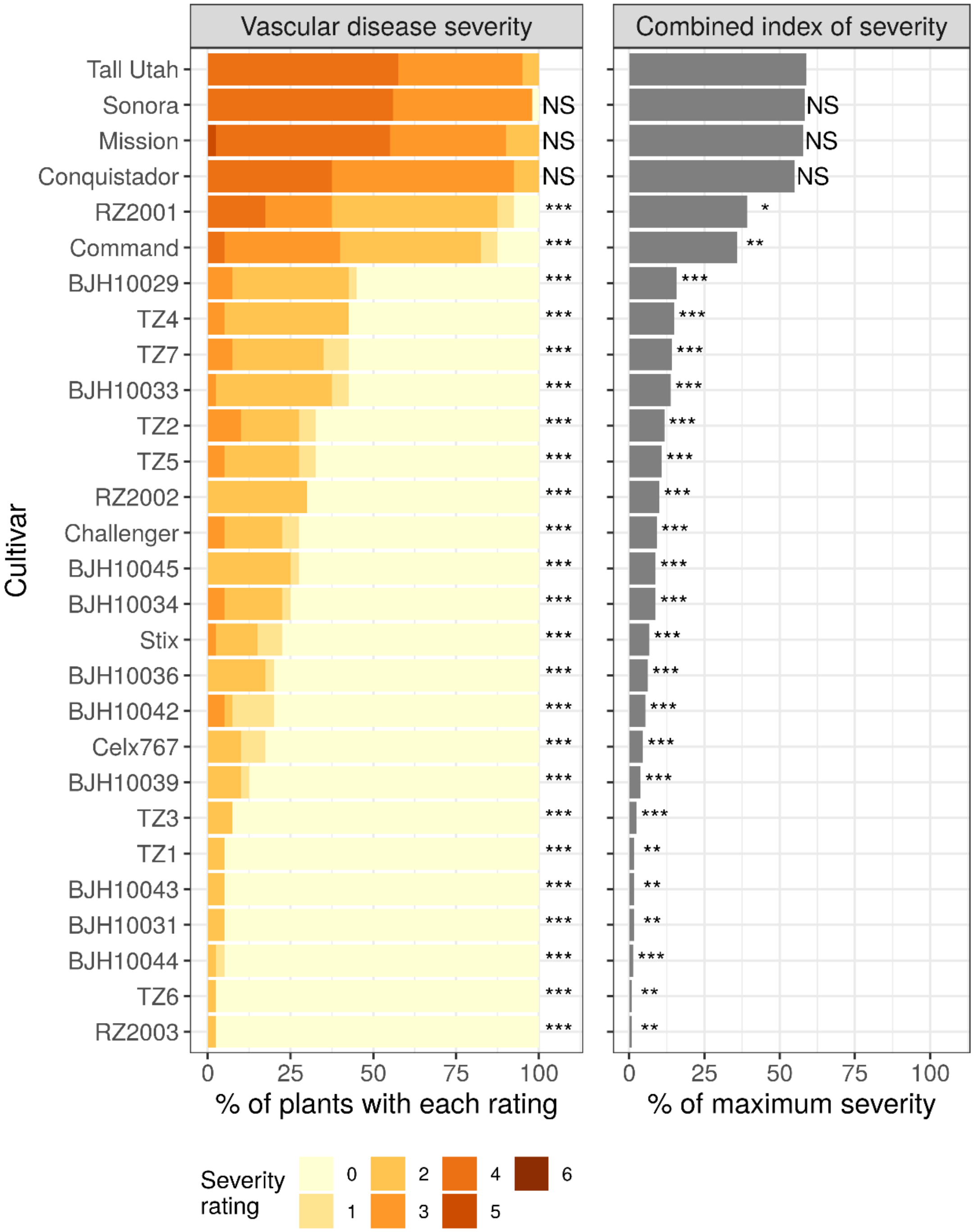

Figure 2: Disease severity at Santa Maria in 2024. Left panel, vascular symptom severity. Right panel, severity summarized as an index. *, **, and *** = significantly different from Tall Utah (susceptible control) at $P \leq 0.05$, 0.01, 0.001 levels, whereas NS indicates no significant difference. Data were derived from 10 plants per plot.

Table 5: Average agronomic scores of cultivar entries in the **Santa Maria FOA2 trial 2024**. The table is sorted alphabetically by cultivar name or germplasm code, not by ranking.

Cultivar	Petiole Length (Inches)	Average Plant Weight (lbs)	Ribbiness (score 1-3)	Pithiness (score 1-3)	Bolting (score 1-3)	Greenness (score 1-3)	Suckering (score 1-3)	Petiole Cracking (score 1-3)
BJH10029	10.6	1.0	2.2	1.2	1.0	2.5	1.5	1.0
BJH10031	11.6	1.1	1.1	1.2	1.0	2.5	1.4	1.0
BJH10033	10.6	1.1	2.3	1.9	1.0	2.8	1.6	1.1
BJH10034	11.6	1.2	1.4	1.6	1.0	2.3	1.8	1.2
BJH10036/Bolero	11.4	1.3	1.1	1.7	1.0	2.3	1.3	1.0
BJH10039/Fandango	11.3	1.4	2.4	1.2	1.0	2.5	1.9	1.1
BJH10042	11.4	1.4	1.3	1.4	1.0	2.2	1.4	1.0
BJH10043/Merengo	11.3	1.3	1.4	2.0	1.0	2.5	1.8	1.0
BJH10044	11.2	1.1	2.0	1.6	1.0	2.3	2.1	1.0
BJH10045/Waltz	10.8	1.0	1.2	1.9	1.0	2.5	1.6	1.1
Celx767	12.0	1.1	2.7	1.2	1.0	2.5	2.1	1.1
Challenger	11.5	1.1	2.0	1.5	1.0	2.5	2.0	1.1
Command	10.6	0.7	1.8	1.4	1.0	2.5	2.7	1.0
Conquistador	6.6	0.2	2.6	1.1	1.0	3.0	2.6	1.1
Mission	6.8	0.2	2.7	1.1	1.0	2.7	2.8	1.2
RZ2001	10.5	0.6	2.5	1.7	1.0	2.6	1.5	1.1
RZ2002	13.7	1.1	2.9	2.1	1.0	2.8	1.1	1.0
RZ2003	15.9	1.6	2.1	1.9	1.0	2.5	2.0	1.0
Sonora	6.2	0.2	2.5	1.3	1.0	3.0	2.3	1.1
Stix	14.4	1.4	2.6	1.6	1.0	2.8	1.9	1.1
Tall Utah	5.8	0.1	2.7	1.0	1.0	3.0	2.7	1.1
TZ1	12.3	1.3	2.0	1.9	1.0	2.8	2.0	1.0
TZ2	11.9	1.3	3.0	1.4	1.0	2.5	1.3	1.1
TZ3	14.1	1.5	2.4	2.0	1.0	2.5	1.8	1.1
TZ4	11.5	1.0	1.6	1.6	1.0	2.4	2.1	1.0
TZ5	11.5	1.2	2.9	1.8	1.0	2.5	1.6	1.0
TZ6	13.2	1.4	2.2	1.6	1.0	2.8	2.4	1.1
TZ7	11.3	1.1	2.7	1.7	1.0	2.5	2.1	1.0

Table 6: Germplasm and cultivar entries in the **Santa Maria FOA2 trial 2024** with no/very mild disease symptoms (disease scores less than 1), low stunting (average petiole length greater than 10 inches), low stunting (average plant weights above 1 lbs), smoother stalks (ribbiness scores less than 2), low pith (pithiness scores less than 2), and low suckering (suckering scores less than 2).

Germplasm/ Cultivar	**Disease Score** **Less than 1**	**Petiole Length** **>10 in**	**Average Plant Weight** **>1 lbs**	**Ribbiness** **Score < 2**	**Pithiness** **Score < 2**	**Suckering** **Score < 2**
BJH10029	x	x	x		x	x
BJH10031	x	x	x	x	x	x
BJH10033	x	x	x		x	x
BJH10034	x	x	x	x	x	x
BJH10036	x	x	x	x	x	x
BJH10039	x	x	x		x	x
BJH10042	x	x	x	x	x	x
BJH10043	x	x	x	x		x
BJH10044	x	x	x		x	
BJH10045	x	x	x	x	x	x
Celx767	x	x	x		x	
Challenger	x	x	x		x	
Command		x		x	x	
Conquistador					x	
Mission					x	
RZ2001		x			x	x
RZ2002	x	x	x			x
RZ2003	x	x	x		x	
Sonora					x	
Stix	x	x	x		x	x
Tall Utah					x	
TZ1	x	x	x		x	
TZ2	x	x	x		x	x
TZ3	x	x	x			x
TZ4	x	x	x	x	x	
TZ5	x	x	x		x	x
TZ6	x	x	x		x	
TZ7	x	x	x		x	

Camarillo FOA4 trial 2024

Methods: There were 38 germplasm and cultivar entries in the race 4 trial from five seed companies, including Bejo Seeds, Diamond Seeds (Spain), Rijk Zwaan, Syngenta, and Tozer Seeds. Seed entries from Diamond Seeds were delayed at APHIS inspection and were planted 5 weeks after the rest of the trial. The transplant date was August 13, 2024. We planted four replications (Reps). Each Rep consisted of the 10 center beds, with unplanted border rows. Reps consisted of 10 beds, each divided into sixteen 15-foot plots. Germplasm entries were randomized within each Rep independently (see map in section Supplemental Materials). The susceptible control was cv. Sonora, and no resistant control was planted. Soil temperatures were recorded at 6 and 12 inches throughout the growing season, however there was a malfunction of the equipment, and the data were not recorded in 2024. Mortality rates were counted on September 20 (week 5). Harvest and disease evaluation took place on December 5+6, 2024 (about 16 weeks after transplant). Pathogen-caused mortality was sufficiently high in most plots that only ten plants were removed from the center of the plot for disease ratings; agronomic measurements and ratings were not taken if there were fewer than 20 plants available. Disease evaluations were conducted by one person per block.

Results: Mortality five weeks after transplant ranged between 1-74% of all entries. BJH10022 and BJH10023 had approximately 1% mortality at five weeks. Both germplasm lines had low disease scores (1.65 and 1.73 respectively), their height at approximately one inch above the average node was near 12 inches (12.5 and 11.43 inches respectively), and they weighed on average about 1.2 lbs. They had moderate/heavy ribs (scores 2.43 and 2.6 out of 3), and had some pith (scores 2.3 and 1.78 out of 3) and some suckering (2.73 and 2.33 out of 3).

The germplasm lines TZ3 and TZ5 had slightly higher mortality rates of 4 and 3% at five weeks. These lines had lower disease scores (1.43 and 1.53 respectively), and their height at approximately one inch above the average node was near 12 inches (13.10 and 11.96 inches respectively), and they weighed on average about 1.95 and 1.48 lbs. They had moderate and heavy ribs respectively (average scores of 1.65 and 2.98 out of 3), and relatively low suckering (average scores of 1.03 and 1.23 out of 3).

Syngenta submissions Celx767 and Stix had mortality of 10% and 30% respectively, and moderate disease scores of 2.68 and 2.15. These are varieties for processing, with average lengths of 12.83 and 12.93 inches approximately one inch above the average node, and weighing on average 1.4 lbs. They have relatively heavy ribs with scores of 2.5 and 2.88 out of 3, low pith scores (average scores of 1.28 out of 3), and suckering scores of 2.5 and 1.5 (out of 3).

Submissions from Rijk Zwaan had average disease scores of 3.95-5.78 (out of 6, in which a score of 6 is plant death), and no agronomics scores were recorded. Submissions from Diamond Seeds were planted 5 weeks after the rest of the field due to delays in the APHIS inspection, and these plants have average disease scores of 3.25-3.88 (out of 6), and no agronomics scores were recorded.

Conclusions: Mortality was lowest in BJH10022 and BJH10023, and disease scores were also low. The average size and weight were also good. TZ3 and TZ5 also had low mortality and low disease scores, with good average size and weight. An increase in the prominence of ribs appears to be a common response to FOA4, and most plants have relatively high scores for ribbiness. Cv. Merengo was included in both the FOA2 and FOA4 trial, and had a ribbiness score of 1.15 out of 3 in the FOA2 trial, and a score of 1.5 out of 3 in the FOA4 trial. It is impossible to make definitive conclusions about the Apio varieties from Diamond Seeds because the plants did not experience the high temperatures soon after transplant, and the plants did not reach full harvest maturity before scoring. Future trials will include more material from this company.

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Table 7: Cultivar entries in **Camarillo FOA4 trial 2024.** Cultivars are arranged alphabetically by breeding company or contributor

Cultivar	Contributor	
BJH10021/Merengo	Bejo Seeds	
BJH10022/Exp 3751	Bejo Seeds	
BJH10023	Bejo Seeds	
BJH10208	Bejo Seeds	
BJH10212	Bejo Seeds	
BJH10218	Bejo Seeds	
Apio21ADS234	Diamond Seeds	
Apio21ADS235	Diamond Seeds	
Apio21ADS236	Diamond Seeds	
Apio242	Diamond Seeds	
Apio243	Diamond Seeds	
Apio244	Diamond Seeds	
Apio245	Diamond Seeds	
RZ4001	Rijk Zwaan	
RZ4002	Rijk Zwaan	
RZ4003	Rijk Zwaan	
RZ4004	Rijk Zwaan	
RZ4005	Rijk Zwaan	
RZ4006	Rijk Zwaan	
RZ4007	Rijk Zwaan	
RZ4008	Rijk Zwaan	
RZ4009	Rijk Zwaan	
Celx767	Syngenta	
Celx767+Cu	Syngenta	
Celx767+KCl	Syngenta	
Command	Syngenta	
Conquistador	Syngenta	
Mission	Syngenta	
Sonora	Syngenta	Susceptible control
Sonora+Cu	Syngenta	
Sonora+KCl	Syngenta	
Stix	Syngenta	
TZ1	Tozer Seeds	
TZ2	Tozer Seeds	
TZ3	Tozer Seeds	
TZ4	Tozer Seeds	
TZ5	Tozer Seeds	
TZ6	Tozer Seeds	

Figure 3: Bar graph of the average percent mortality among replications in 2024.

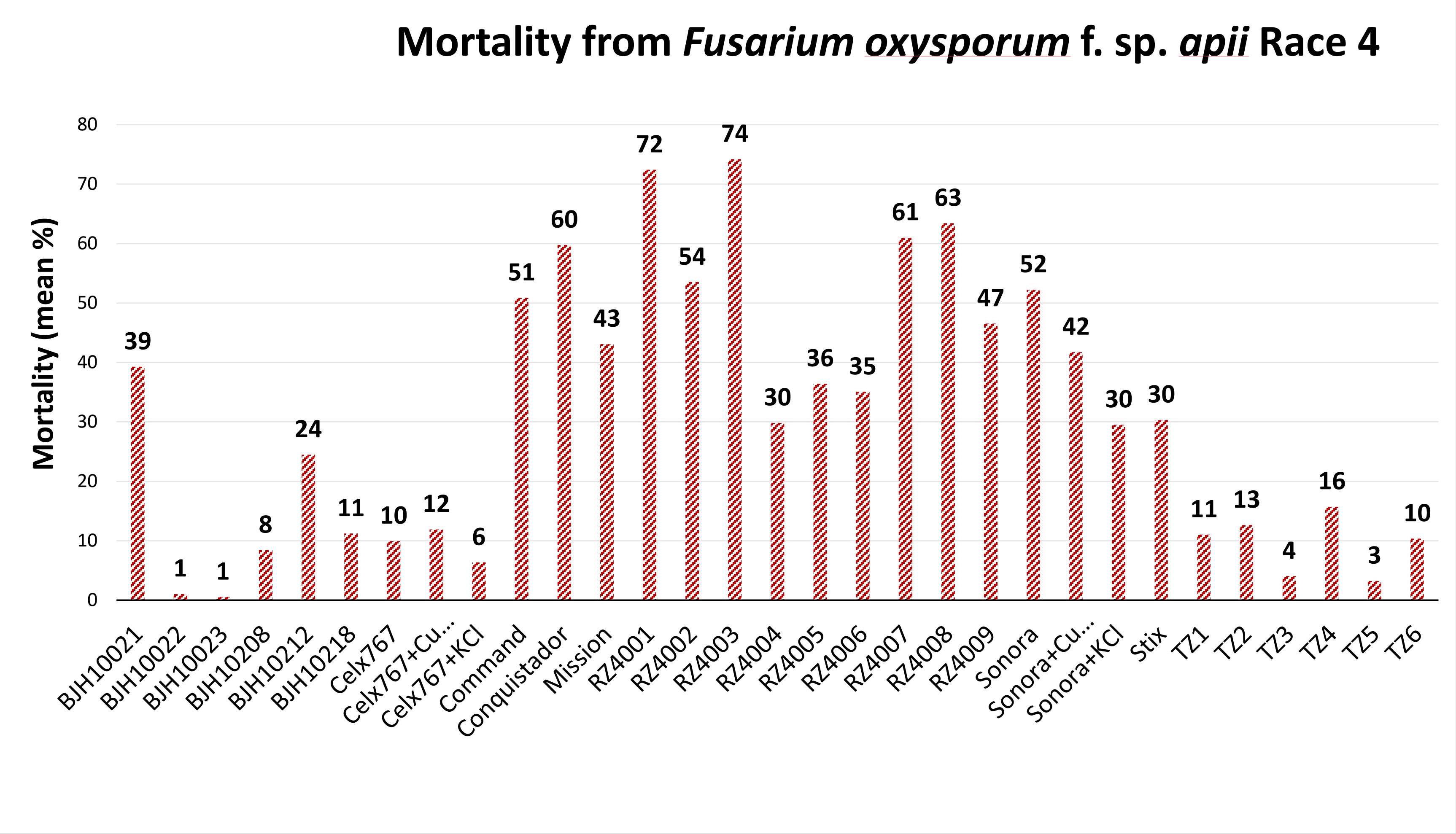


Table 8: Disease rating scores and mortality of cultivar entries in **Camarillo FOA4 trial 2024.** Cultivars are arranged from low to high disease rating scores

Cultivar	Average Disease Score (0-6)	Mortality (Percent of Total Planted)
TZ3	1.43	4
TZ5	1.53	3
BJH10022	1.65	1
BJH10023	1.73	1
TZ2	1.93	13
Stix	2.15	30
TZ1	2.33	11
BJH10021 or Merengo	2.43	39
TZ6	2.55	10
Celx767	2.68	10
TZ4	2.75	16
BJH10218	2.83	11
BJH10208	3.13	8
Apio21ADS234	3.25	NA
Command	3.48	51
Apio245	3.48	NA
BJH10212	3.50	24
Apio242	3.63	NA
Apio244	3.69	NA
Apio21ADS236	3.85	NA
Apio243	3.88	NA
RZ4006	3.95	35
Apio21ADS235	4.18	NA
RZ4004	4.28	30
RZ4005	4.53	36
Conquistador	5.43	60
Mission	5.60	43
RZ4001	5.60	72
RZ4009	5.68	47
Sonora	5.68	52
RZ4007	5.70	61
RZ4008	5.75	63
RZ4002	5.78	54
RZ4003	5.80	74

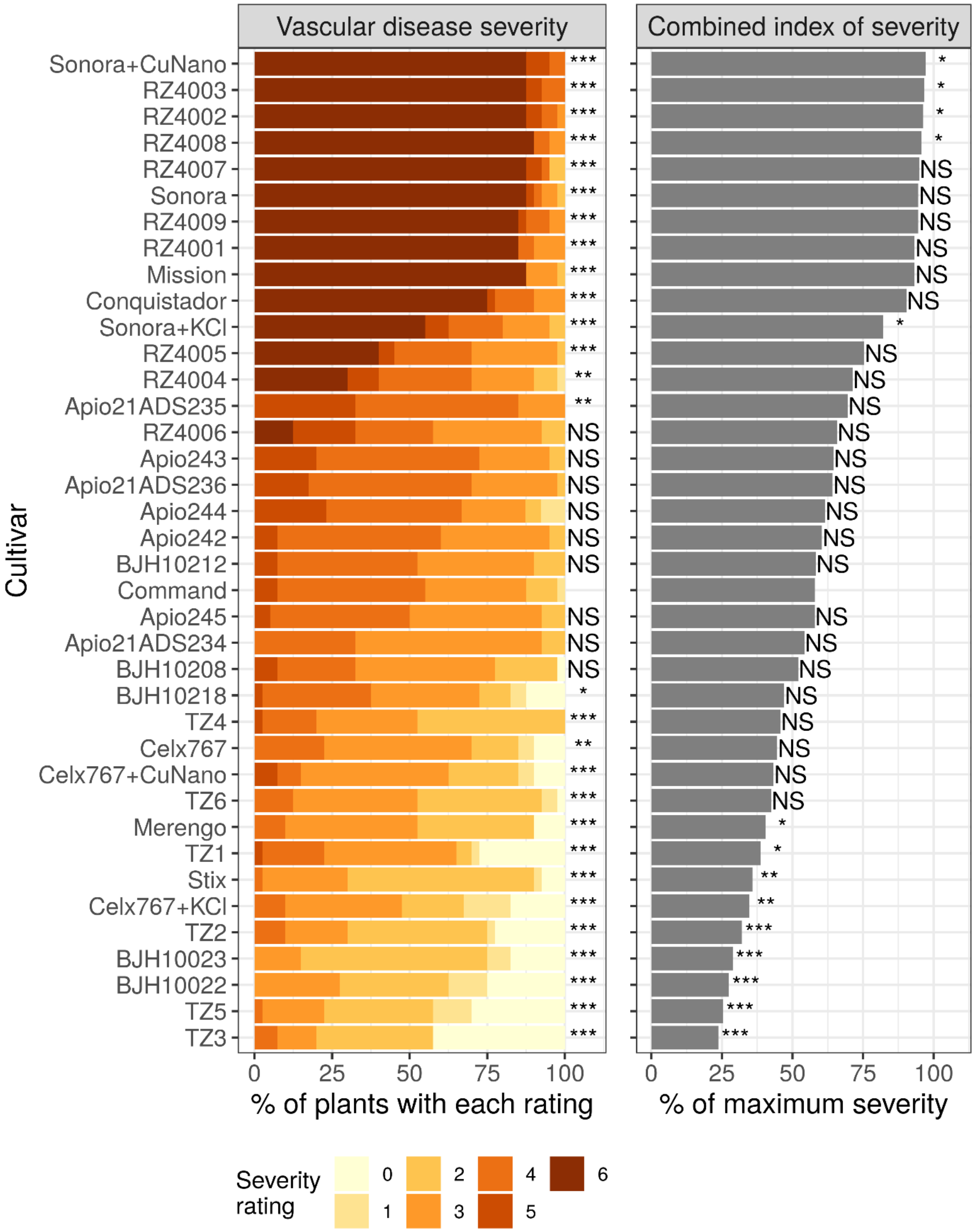


Figure 4. Disease severity at Camarillo in 2024. Left panel, vascular symptom severity. Right panel, severity summarized as an index. *, **, and *** = significantly different from **Command** at $P \leq 0.05$, 0.01, 0.001 levels, whereas NS indicates no significant difference. Data were derived from 10 plants per plot.

Table 9: Average agronomic scores of cultivar entries in the **Camarillo FOA4 trial 2024**. Table is sorted alphabetically by cultivar name or code. NA indicates that too many of the plants were dead to rate. No agronomic data were taken for accessions from Diamond Seeds due to the late transplant or from Rijk Zwaan due to plant death.

Cultivar	**Petiole Length (inches)**	**Average Plant Weight (lbs)**	**Ribbiness (score 1-3)**	**Pithiness (score 1-3)**	**Bolting (score 1-3)**	**Greenness (score 1-3)**	**Suckering (score 1-3)**	**Petiole Cracking (score 1-3)**
BJH10022/Exp 3751	12.5	1.2	2.4	2.3	1.0	2.0	2.7	1.1
BJH10023	11.4	1.3	2.6	1.8	1.0	2.0	2.3	1.0
TZ5	12.0	1.5	3.0	1.3	1.0	3.0	1.2	1.1
TZ3	13.1	2.0	1.7	1.1	1.0	2.9	1.0	1.1
BJH10208	6.7	0.5	3.0	1.0	1.2	2.9	1.5	1.3
TZ6	9.7	0.8	3.0	1.1	1.0	2.8	1.5	1.3
Celx767	12.8	1.4	2.5	1.3	1.0	2.6	2.5	1.2
TZ1	11.6	1.6	2.4	1.1	1.0	2.9	1.2	1.1
BJH10218	8.5	1.1	1.2	1.2	1.0	2.7	1.1	1.2
TZ2	11.3	1.4	2.9	1.5	1.0	3.0	1.2	1.2
TZ4	9.5	1.0	2.6	1.6	1.0	2.6	1.8	1.3
BJH10212	6.0	0.3	2.7	1.0	1.5	2.7	2.0	1.3
Stix	12.9	1.4	2.9	1.3	1.0	2.8	1.5	1.2
RZ4004								
RZ4006								
RZ4005								
BJH10021/Merengo	10.3	1.4	1.5	1.3	1.0	2.8	2.0	1.0
Mission								
RZ4009								
Command	7.5	0.7	3.0	1.0	1.0	3.0	2.2	1.0
Sonora								
RZ4002								
Conquistador								
RZ4007								
RZ4008								
RZ4001								
RZ4003								
Apio21ADS234								
Apio245								
Apio242								
Apio244								
Apio21ADS236								
Apio243								
Apio21ADS235								

Table 10: Germplasm and cultivar entries in the **Camarillo FOA4 trial 2024** with mild disease symptoms (disease scores less than 2), low mortality (less than 10%), low stunting (average petiole length greater than 10 inches), low stunting (average plant weights above 1 lbs), smoother stalks (ribbiness scores less than 2), low pith (pithiness scores less than 2), and low suckering (suckering scores less than 2).

Germplasm/ Cultivar	Disease Score Less than 2	Mortality Less than 10%	Petiole Length > 10 in	Average Plant Weight >1 lbs	Ribbiness Score < 2	Pithiness Score < 2	Suckering Score < 2
Apio21ADS234							
Apio21ADS235							
Apio21ADS236							
Apio242							
Apio243							
Apio244							
Apio245							
BJH10021/Merengo			x	x	x	x	
BJH10022/Exp 3571	x	x	x	x			
BJH10023	x	x	x	x		x	
BJH10208		x				x	x
BJH10212						x	
BJH10218				x	x	x	x
Celx767		x	x	x		x	
Command						x	
Conquistador							
Mission							
RZ4001							
RZ4002							
RZ4003							
RZ4004							
RZ4005							
RZ4006							
RZ4007							
RZ4008							
RZ4009							
Sonora							
Stix			x	x		x	x
TZ1			x	x		x	x
TZ2	x		x	x		x	x
TZ3	x	x	x	x	x	x	x
TZ4						x	x
TZ5	x	x	x	x		x	x
TZ6		x				x	x

Objective 3: Evaluate performance of a susceptible cultivar with additional micronutrient amendments

Within the Camarillo FOA4 trial, we placed additional plots of Sonora (FOA4 susceptible) and Celx767 (FOA4 relatively tolerant) treated with potassium chloride (KCl) and copper (Cu) nanoparticles. Transplants were partially submerged in a root drip solution of 10% KCl, and a 0.5% Cu nanoparticle solution treated with a solvent. They were handplanted and maintained in the same manner as the remainder of the field trial.

Fusarium disease scores for susceptible Sonora plants were statistically lower (better) when KCl was applied (ANOVA and THSD F = 9.4, P = 0.003), however the scores were still high, and mortality was 30% following transplant. Disease scores for Sonora plants treated with copper nanoparticles were not statistically different than untreated Sonora plants. In Celx767, which appears more tolerant of FOA4 than Sonora, there was no statistical difference in disease scores for plants treated or untreated with copper nanoparticles or KCl (ANOVA F = 2.76, P = 0.07).

Conclusion: There was no practical effect of treating roots pre-transplant with potassium chloride or copper nanoparticles under these experimental conditions.

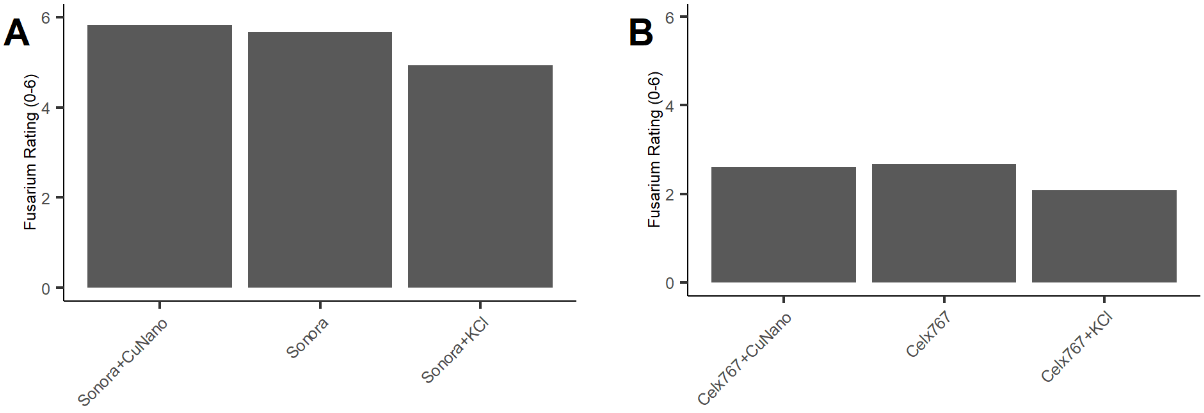


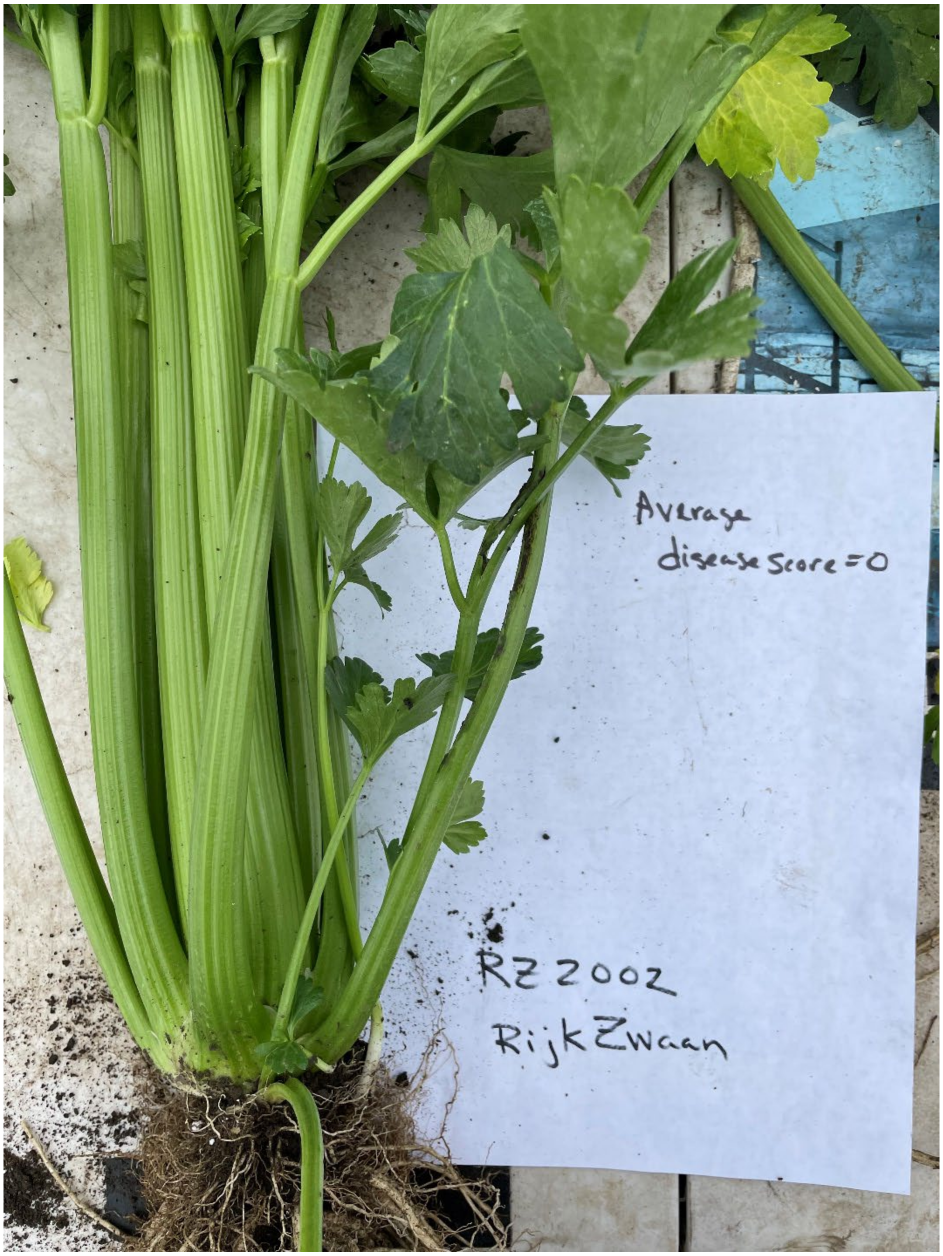
Fig. 5: Fusarium disease scores for Sonora (A) and Celx767 (B). Sonora had higher (worse) disease scores overall, and a root dip of potassium chloride solution improved disease scores in the roots and crown, however, did not improve agronomic tolerance to FOA4.

Objective 4: Outreach events

1. We hosted two field days on September 19 (Santa Maria) and December 6 (Camarillo).
2. We published a short article in the CAPCA industry newsletter. Daugovish, O., C. Greer, R. L. Eriksen, and Alex Putman. 2024. Fusarium yellows in celery: identification and management. CAPCA Adviser. XXVII(1), 37-39.
3. The in-progress results were presented by Daugovish at the 2024 CAPCA meeting in Ventura County, Feb 22, 2024. (73 attendees) and
4. by Daugovish at an industry pest management meeting Jan. 10 at Camarillo, CA (61 attendees)

5. Eriksen presented research from these trials at the Salinas Valley Pest Management Task Force meeting, organized and run by the Grower Shipper Association, on June 20, 2024 (12 attendees).
6. Greer presented research at the American Society for Horticultural Science meeting. Greer, C., O. Daugovish, R. Eriksen, and A. Putman. 2024. Evaluation of Celery Breeding Lines and Commercial Cultivars for Resistance to Fusarium oxysporum f.sp. apii Race 2 and Race 4. HortScience: Abstracts of Presentations from the 2024 Annual Conference of the American Society for Horticultural Science. 59:S9, S455. October 12, 2024. (>1,000 attendees)

Photos from **Santa Maria FOA2 trial 2024**


Average
disease score = 0
RZ 2002
RijkZwaan


Average
isease Score =0
TZ4
zer Seeds


Average
disease sc
TALL UTAH
susceptible


Conquistador
Syngenta Seeds
Sila
Syngenta Seeds
TZ5
Tozer Seeds
TZ5
Tozer Seeds


Command
Syngenta Seeds

Photos from **Camarillo FOA4 trial 2024**

Photos taken on September 30, 2024

BJH10022/Exp3751

Celx767

Mission

RZ4008

TZ1

TZ3

CALIFORNIA CELERY RESEARCH ADVISORY BOARD ANNUAL REPORT

Project title: Diagnostic test for identification and quantification of *Fusarium oxysporum* f. sp. *apii* race 4 in soil and plant material

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Abstract:

The soilborne disease fusarium wilt of celery is a significant threat to commercial celery production in California. Fusarium wilt of celery is caused by the fungus *Fusarium oxysporum* f. sp. *apii*, which contains multiple races (i.e., pathogen types). Among these races, *F. oxysporum* f. sp. *apii* (*Foa*R4) is the greatest issue in celery production due to its increased virulence and ability to overcome sources of genetic resistance used to control other races. Sustainable management of disease caused by *Foa*R4 requires knowledge of what fields are infested with this pathogen and the ability to identify and measure *Foa*R4 in plant and soil samples. The primary goal of this project is to develop a sensitive and accurate molecular diagnostic test that can be used to detect and quantify *Foa*R4 in soil and plant material. Genetic loci for the diagnostic test will be identified by comparing genomic data from *Foa*R4 to a genome sequence database composed of 750 *F. oxysporum* isolates, including representatives from known *F. oxysporum* f. sp. *apii* races and closely related pathogen *F. oxysporum* f. sp. *coriandrii* (*Foc*). Target diagnostic locus or loci specific for *Foa*R4 will be used to design primers and probes for a quantitative polymerase chain reaction (qPCR)-based molecular diagnostic test. Accuracy and sensitivity of the diagnostic test will be evaluated using a panel of DNA from *F. oxysporum* f. sp. *apii* races, *Foc*, and additional pathogenic and non-pathogenic forms of *F. oxysporum* commonly found in the environment. The test will be further validated by testing soil from celery fields with a known presence *Foa*R4 and celery plant material from the USDA-ARS celery breeding program. Outcomes of this research will provide growers with a tool to confidently test if plants are infected with *Foa*R4, identify fields that are infested with this pathogen, and determine if disease management strategies are reducing *Foa*R4 inoculum in soil.

Objectives:

- Objective 1. Identify genetic loci that can differentiate *Foa*R4 from other *F. oxysporum* f. sp. *apii* races and non-target pathogens
- Design and optimize a molecular diagnostic test for *Foa*R4 that enables detection and quantification of *Foa*R4 in plant material and soil samples.

Procedures:

Objective 1. Identify genetic loci that can differentiate *Foa*R4 from other *F. oxysporum* f. sp. *apii* races and non-target pathogens

Genome assemblies of *Foa*R4 were compared to a custom database of 727 additional non-target fungi to identify genetic loci that are unique to *Foa*R4 and could serve as a platform for molecular diagnostic tests. The custom database includes representatives that are closely related to *Foa*R4 and potentially found in the same plant or soil sample, such as *Foc* and nonpathogenic/saprophytic *F. oxysporum*. Analyses were conducted by aligning raw genomic sequence data from *Foa*R4 to the custom database to identify raw sequences that are unique to *Foa*R4. These raw sequences unique to *Foa*R4 were assembled into new longer DNA sequences (i.e., contigs). Raw sequence data from non-target fungi then were mapped back to the new *Foa*R4 contigs to develop a list of candidate genetic loci that would serve as diagnostic markers for *Foa*R4. These analyses provided a list of genetic loci that were 1) present in all genome assemblies of *Foa*R4 2), could differentiate this pathogen from the closely related pathogen *Foc*, and 3) were present in the fewest number of non-target fungi.

Objective 2. Design and optimize a molecular diagnostic test for *Foa*R4 that enables detection and quantification of *Foa*R4 in plant material and soil samples

A molecular test called polymerase chain reaction (PCR) was used to further validate the candidate *Foa*R4 genetic loci identified in Objective 1. PCR primers were designed based on the underlying DNA sequence data to introduce specificity for *Foa*R4 and exclude non-target fungi.

Genomic DNA was extracted from a panel of 25 target and non-target fungal isolates obtained from collaborators and culture collections. Newly designed PCR primers were tested against the DNA samples to determine which primers and genetic loci produced true positives (i.e., PCR detection of *Foa*R4 isolates) versus false positive reactions (e.g., PCR detection of non-target fungi like *Foc*). As PCR amplification specificity is temperature dependent, reactions were performed using temperature gradients for all primer sets. These PCR reactions were also used to identify which primers produced a single band (e.g., see Figure 1 below), indicating an absence of non-specific PCR amplification.

Results:

Objective 1: Bioinformatic analysis of genomic data from over 700 fungal isolates, including *Foa*R4, enabled identification of 13 candidate genetic loci with potential use in molecular diagnostic tests. After reviewing the 13 candidate genetic loci, 7 were identified further validation using PCR. Genetic loci were considered good candidates based on their presence in *Foa*R4 and complete absence in *Foc*. A summary of the 7 genetic loci and their presence in non-target isolates is provided in Table 1.

Table 1. Summary of five candidate genetic loci for diagnostic testing of *Foa*R4 identified in Objective 1 and used for PCR testing in Objective 2. The genetic locus selected for use in designing additional molecular diagnostic tests is highlighted in bold.

Locus no.[a]	**Number of non-targets**[b]	***Foc* PCR detection**[c]	***Foa*R4 PCR detection**[d]
13	13	no	yes
19	15	yes	no
21	13	no	yes

29	13	yes	yes
37	**4**	**no**	**yes**
50	13	no	yes
75	13	no	yes

a. Genetic locus number identified using bioinformatic methods in Objective 1.
b. Number of non-target fungal isolates (e.g., nonpathogenic *F. oxysporum* or non-celery pathogen) that have the genetic locus in its genome.
c. Results from PCR testing (Objective 2) indicating that non-target *Foc* samples produced false positive results.
d. Results from PCR testing (Objective 2) showing consistent positive detection of target samples of *Foa*R4. Examples of consistent PCR amplification are shown in Figure 1 below.

Objective 2: A total of 47 PCR primers were tested using the panel of 25 DNA samples from target and non-target fungal isolates. These primers were designed for 7 of the 13 original candidate genetic loci identified in Objective 1. As shown in Table 1, two genetic loci gave false positive results from *Foc*, even though the genomic data suggested that the loci were absent in that pathogen. Another single locus did not give consistent positive amplification of the targeted *Foa*R4 samples. Based on these data, a single genetic locus and PCR reaction temperature was identified that provides consistent PCR amplification *Foa*R4 and limited detection of non-targets, including the closely related pathogen *Foc*. The one potential non-target that is detected by using this genetic locus is a single historical representative of *F. oxysporum* f. sp. *apii* Race 3 from the 1980s, which is no longer documented in the environment and unlikely to cause false positive tests from contemporary field samples (Figure 1 and Table 1).

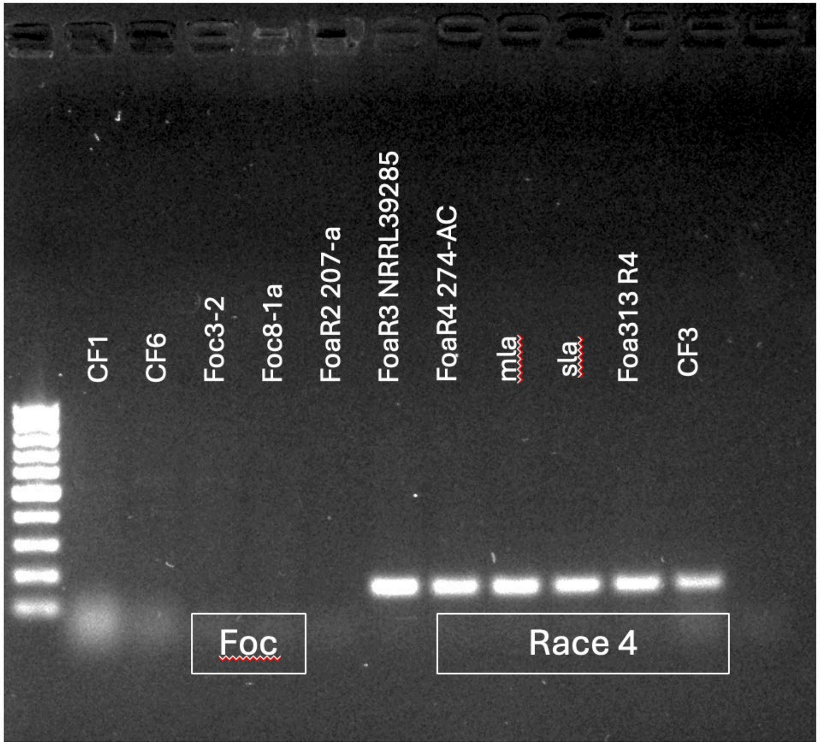

Figure 1. PCR detection using diagnostic primers for *Fusarium oxysporum* f. sp. *apii* race 4 (*Foa*R4) for locus 37. Samples are labelled in white text (CF1 to CF3). A white band indicates the PCR successfully amplified/detected a sample. All *Foa*R4 samples were detected. Non-target samples were not detected, except the historical sample of race 3 (FoaR3 NRRL39285). "Foc" shows samples from *F. oxysporum* f. sp. *coriandrii*.

Discussion:

The soilborne pathogen *Fusarium oxysporum* f. sp. *apii* Race 4 (*Foa*R4), causal agent of Fusarium wilt of celery, is a significant problem in California celery production. The ability of *Foa*R4 to kill disease resistant plants, survive in soil, and infect non-celery crops add a unique set of challenges for managing Fusarium wilt disease. Long-term control of *Foa*R4 and Fusarium wilt of celery partially depends on proper identification and quantification of the pathogen in plant and environmental samples. Unfortunately, there are limited diagnostic tools that enable growers or disease clinics to properly identify and measure *Foa*R4. This project aims to overcome this limitation by developing molecular diagnostic tools for *Foa*R4.

The first year of this new project focused on identifying genetic loci (i.e., DNA sequences) that could serve as a platform for longer-term development of molecular diagnostic tools for *Foa*R4. A primary challenge in identifying these genetic loci is that *Foa*R4 is genetically very similar to other types of *F. oxysporum* that do not cause disease on disease resistant celery plants, such as *F. oxysporum* f. sp. *coriandrii* (*Foc*). By analyzing genomic data from *Foa*R4 and hundreds of non-target fungi, we identified several genetic loci that are present in *Foa*R4 and absent in *Foc* (Table 1). These genetic loci were also largely absent in other fungi that are common in the environment making them good candidates for molecular diagnostic tests for the target pathogen.

The genetic loci identified in *Foa*R4 using genomic data were further tested using a method called PCR. This molecular method allows us to test whether the genetic locus is present in a DNA sample from a fungus. By applying this method to DNA samples that were previously identified as *Foa*R4, *Foc*, or other non-target fungi we narrowed down the list of candidate genetic loci identified in Objective 1. The genetic locus number 37 was the best candidate since it showed consistent positive detection of *Foa*R4, did not show false positive detection of *Foc*, and was present in the fewest number of non-target fungi (Table 1 and Figure 1). It should be noted that locus number 37 is present in 4 non-target fungi. However, the reaction conditions and PCR primers were designed 3 of the 4 non-target fungi are not detected. The one potential false positive is from a single historical sample of *F. oxysporum* f. sp. *apii* Race 3 collected in the 1980s. As this is the only documentation of Race 3, it is highly unlikely this pathogen is present in the environment and will pose limited risk of producing false positive reactions when testing contemporary samples.

The outcomes of this year's research provide a new genetic locus that can differentiate *Foa*R4 from the closely related pathogen *Foc* and can serve as a platform for designing molecular diagnostic tools. Looking forward, this project will build on these data to design and validate two molecular diagnostic tests for *Foa*R4. The first one will be a qPCR test that enables measurement of *Foa*R4 in plant and environmental samples. This quantitative test could be useful for determining pathogen inoculum load in a field or plant sample, but requires several hours to process samples and get results. The second test will be a recombinase polymerase assay (RPA) which can be used for rapid testing of crushed up plant material with relatively fewer laboratory equipment. Further application of these tools will help provide information on the frequency of *Foa*R4 in CA celery and whether efforts to mitigate Fusarium wilt disease are effective.

California Celery Research Advisory Board
Annual Report for 2023-2024
Management and Economics of Insect Pests on Celery

LEE-24

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Immediate Objectives:

I. Studies to minimize the negative effects of FQPA: Identifying control ‘gaps’ and finding solutions. Immediate goals are to screen new insecticides for insect control and resistance management programs on celery.

II. Continue economic analyses of IPM approaches developed through the support of the CCRAB using new insecticide chemistries. A comparison between the IPM and a "standardized" chemical approach has been evaluated.

Objective I. Screen new insecticides for insect control

Celery, variety "Merengo", was transplanted on 21 Sep 2023 at Agricultural Operations, University of California, Riverside, California (Figure 1A). The plots were three beds wide (two rows per bed on 60" centers) by 40 feet and were drip irrigated. Four replicates of each treatment were organized in a randomized complete block design. In addition to a non-treated control, the remaining treatments were foliar applications of Beleaf 50 SG, VST Exp. combined with Dipel DF, a rotation of VST Exp. combined with Dipel DF and Exirel 100 SE combined with Sequoia SC, an organic rotation, a low input IPM rotation, and Mustang Maxx. The organic rotation included Aza-direct, Entrust SC, Pyganic 5.0 EC, Trilogy EC, Dipel DF, Grandevo WDG, and Venerate XC along with Oroboost as an adjuvant at 0.25%. The low input treatment consisted of Exirel 100SE, Radiant 1.0 SC, Sequoia SC, and Dipel DF (Table 1). The VST exp. treatments used Dyne-amic as an adjuvant at 0.125%, and 0.25% Dyne-amic was used with the low input, Beleaf, and Mustang Maxx treatments. Treatments and spray dates are listed in Table 1.

In our studies, all chemicals were applied by tractor using a tractor-mounted boom sprayer operated at 100 PSI and 100 gal/Ac (Figure 1B). Four, disc-type cone nozzles per bed incorporated D3 orifice disks, #25 cores, and 50 mesh screens.

Table 1. IPM Materials Evaluated:

Compound [b]	Formulation	Rate/Ac	MoA [a]	Supplier
1- Control-non-treated	--	--	--	--
2- Beleaf – foliar	50 SG	2.8 oz	9C	FMC
Dyne-amic		0.25%		
(11/16, 12/1, 1/10)				
3- VST-Exp. +		8 oz	--	Vestaron
Dipel	DF	0.5 lb	11	Valent
Dyne-amic		0.125%		Loveland
(11/16,11/21,12/1,12/7, 12/13,1/10)				
4- a,c-VST-Exp. +		8 oz	--	Vestaron
Dipel	DF	0.5 lb	11	Valent
b-Exirel	100 SE	13.5 oz	28	FMC
+ Sequoia	SC	5.0 oz	4C	Corteva
d-Dipel	DF	1 lb	11	Valent
Dyne-amic		0.125%		Loveland
(11/16, 11/21, 12/7,12/13,1/10)				
5- Organic IPM				
a-Aza-direct		32 oz	UN	Gowan
b-Entrust	SC	8.0 oz	UNB	Corteva
c-Pyganic +	5.0EC	17.0 oz	3A	MGK
Trilogy (1%)	EC	128.0 oz	UN	Certis
d-Dipel	DF	1 lb	11	Valent
e-Grandevo	WDG	3 lb	UNB	Marrone Bio
f-Venerate	XC	128.0 oz	UNB	Marrone Bio
Oroboost		0.25%		Oro-Agri
(11/16,11/21,12/1,12/7, 12/13,1/10)				
6- IPM				
a-Exirel	100 SE	13.5 oz	28	FMC
+ Sequoia	50 SG	2.8 oz	4C	Corteva
b-Radiant	SC	8.0 oz	5	Corteva
+ Sequoia	50 SG	2.8 oz	4C	Corteva
c-Exirel	100 SE	13.5 oz	28	FMC
d-Dipel	DF	1 lb	11	Valent
Dyne-amic		0.25%		Loveland
(11/16,12/1,12/7,1/10)				
7- Mustang Maxx-foliar	0.8 EC	4.0 oz	3A	FMC
Dyne-amic		0.25%		Loveland
(11/16,11/21,12/7,12/13,1/10)				

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[a] MoA – Mode of Action for insecticide resistance management

[b] Spray dates are included.

Figure 1. A is a celery field plot at UCR Agricultural Operations in 2023-2024. B is the tractor setup for commercial scale application.

IRAC

Using the guidelines established by the Insecticide Resistance Action Committee (IRAC) we are classifying the compounds used in the field trial based on their mode of action (MoA) (see Table 1). The purpose is to assist growers in the development of insecticide resistance management (IRM) strategies. These strategies will improve the effectiveness of IPM programs, slow resistance development, and extend the life of the pesticides. The following language is from the IRAC; additional information and MoA classifications can be found in the IRAC handout or online at www.irac-online.org.

> "Resistance to insecticides may be defined as '*a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species*' (IRAC). This definition differs slightly from others in the literature, but IRAC believes it represents the most accurate, practical definition of relevance to farmers and growers. Resistance arises through the over-use or mis-use of an insecticide or acaricide against a pest species and results in the selection of resistant forms of the pest and the consequent evolution of populations that are resistant to that insecticide or acaricide.
>
> In the majority of cases, not only does resistance render the selecting compound ineffective but it often also confers cross-resistance to other chemically related compounds. This is because compounds within a specific chemical group usually share a common target site within the pest, and thus share a common mode of action (MoA). It is common for resistance to develop that is based on a genetic modification of this target site. When this happens, the interaction of the selecting compound with its target site is impaired and the compound loses its pesticidal efficacy. Because all compounds within the chemical sub-group share a common MoA, there is a high risk that the resistance that has developed will automatically confer cross-resistance to all the compounds in the same sub-group. It is this concept of cross-resistance within chemically related insecticides or acaricides that is the basis of the IRAC mode of action classification".

Field Counts:

The field was surveyed on 10 January for Lepidopteran pests (primarily beet armyworm, (*Spodoptera exigua* (Hübner)) and cabbage looper (*Trichoplusia ni* (Hübner)), leafminer (*Liromyza trifolii*) (Burgess), cabbage aphid (*Brevicoryne brassicae*)(Linnaeus), lygus bug (*Lygus hesperus*)(Knight), greenhouse whitefly (*Trialeurodes vaporariorum*)(Westwood), and earwigs (*Forficula auricularia*) (De Geer) (Figure 2). Five plants from each plot were counted for a total of twenty plants per treatment. In addition to insect presence, insect damage was also assessed.

Of the insects listed we found aphids, Lygus bug, earwigs, and beet armyworm, in numbers sufficient to analyze. Statistical differences between treatments were seen for beet armyworm (Leps), lygus bug, and earwigs on 10 January (Figure 3). All lepidopteran treatments performed well with less than 2.5 percent damage. Aphid numbers were highest in the organic treatment plots. Significant differences were observed for lygus and earwigs, but the overall pest pressure was moderate. Heavy periods of rain were present throughout the growing season.

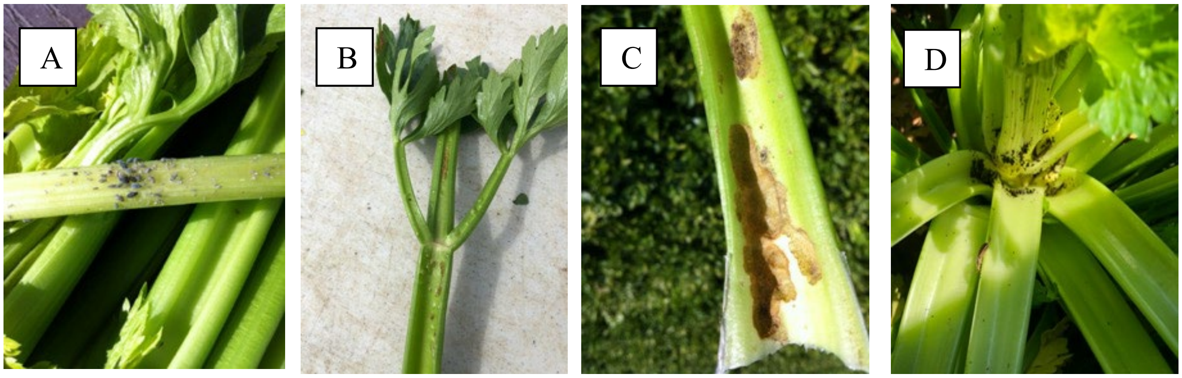
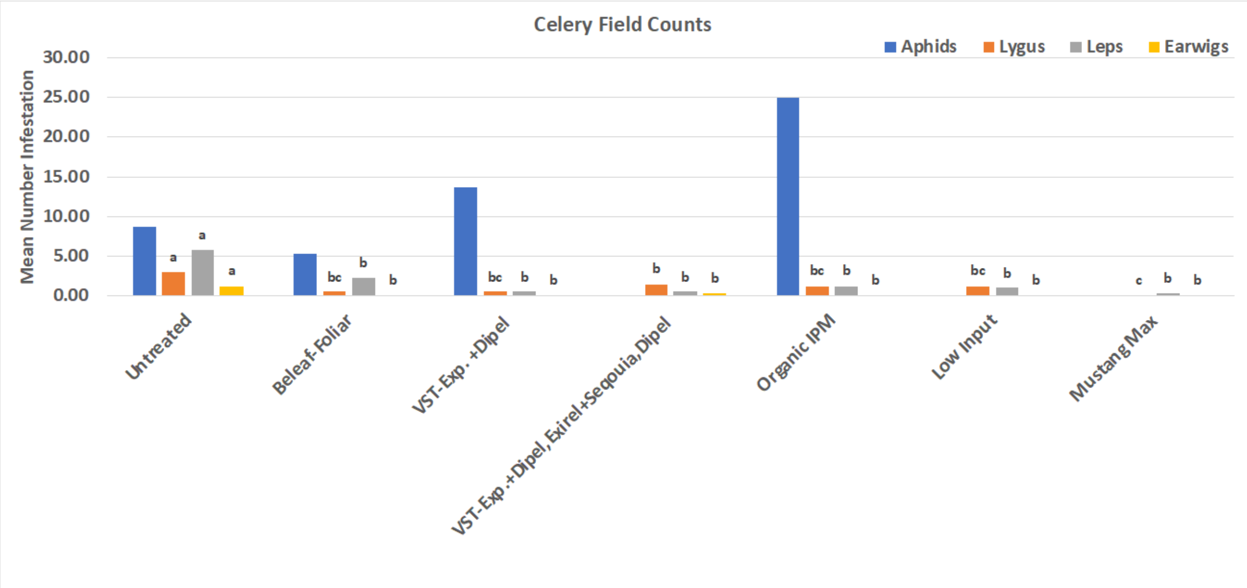


Figure 2. Insects and damage seen in the Celery IPM Trials at the UCR Agricultural Operations research study. A. Aphids; B. Lygus bug damage; C. Beet armyworm damage; D. Earwig damage.

Figure 3. Field counts level of infestation on 10 January (insects plus damage)



Harvest Evaluations:

The field was evaluated at harvest on 31 January. The number of damaged plants found in 25 plants per replicate (4 replicates/treatment) from the center rows of each replicate were counted and recorded. Beet armyworm densities were high in the study; the control suffered 33% (8.25/25 plants) damage (Table 2). There were significant differences between the treatments for worm control. The IPM rotation of Exirel, Sequoia, Radiant, and Dipel performed the best with 0% damage. The rotation of VST, Dipel, Exirel, and Sequoia also performed well with 2% damage. Cutworm pressure was very low this season. Leafminers and whiteflies have not been included because of low pest pressure. Similarly, while a few earwigs and lygus were seen in our field counts, there was very little damage on the plants at harvest. Cabbage aphid was present in the field this year, and there were significant differences between the treatments. The best performing products were the IPM rotation of Exirel, Sequoia, Radiant, and Dipel with 0% damage. The Mustang Maxx treatment also had 0% damage. Lygus bug pressure was high and significant differences were seen between the treatments. The best performing product was Mustang Maxx. No phytotoxicity was observed in any of the treatments.

TABLE 2. HARVEST MEAN NUMBER OF PLANTS DAMAGED

		Mean No. Plants Infested or Damaged by [a]		
Treatment/Spray Dates	**Rate**	**Aphids**	**Beet Armyworm**	**Lygus Bug**
1- Control-Non-treated	--	6.00 a	8.25 a	12.50 a
2- Beleaf – foliar Dyne-amic (11/16, 12/1, 1/10)	2.8 oz 0.25%	2.25 b	3.75 b	6.00 b
3- VST-Exp. + Dipel Dyne-amic (11/16,11/21,12/1,12/7, 12/13,1/10)	8 oz 0.5 lb 0.125%	2.25 b	2.00 b	7.75 b
4- a,c-VST-Exp. + Dipel b-Exirel + Sequoia d-Dipel Dyne-amic (11/16, 11/21, 12/7,12/13,1/10)	8 oz 0.5 lb 13.5 oz 5.0 oz 1 lb 0.125%	2.25 b	0.50 b	6.25 b
5- Organic IPM a-Aza-direct b-Entrust c-Pyganic + Trilogy (1%) d-Dipel e-Grandevo f-Venerate Oroboost (11/16,11/21,12/1,12/7, 12/13,1/10)	 32 oz 8.0 oz 17.0 oz 128.0 oz 1 lb 3 lb 128.0 oz 0.25%	8.25 a	3.75 b	6.25 b

6- IPM a-Exirel + Sequoia b-Radiant + Sequoia c-Exirel d-Dipel Dyne-amic (11/16,12/1,12/7,1/10)	13.5 oz 2.8 oz 8.0 oz 2.8 oz 13.5 oz 1 lb 0.25%	0.00 b	0.00 b	4.75 bc
7- Mustang Maxx-foliar Dyne-amic (11/16,11/21,12/7,12/13,1/10)	4.0 oz 0.25%	0.00 b	3.00 b	1.50 c
ANOVA F_{value}		0.001	0.007	0.002
ANOVA P value		8.290	4.159	5.338

[a] Means in columns followed by the same letter are not significantly different ($P < 0.05$, Fisher's Protected LSD).

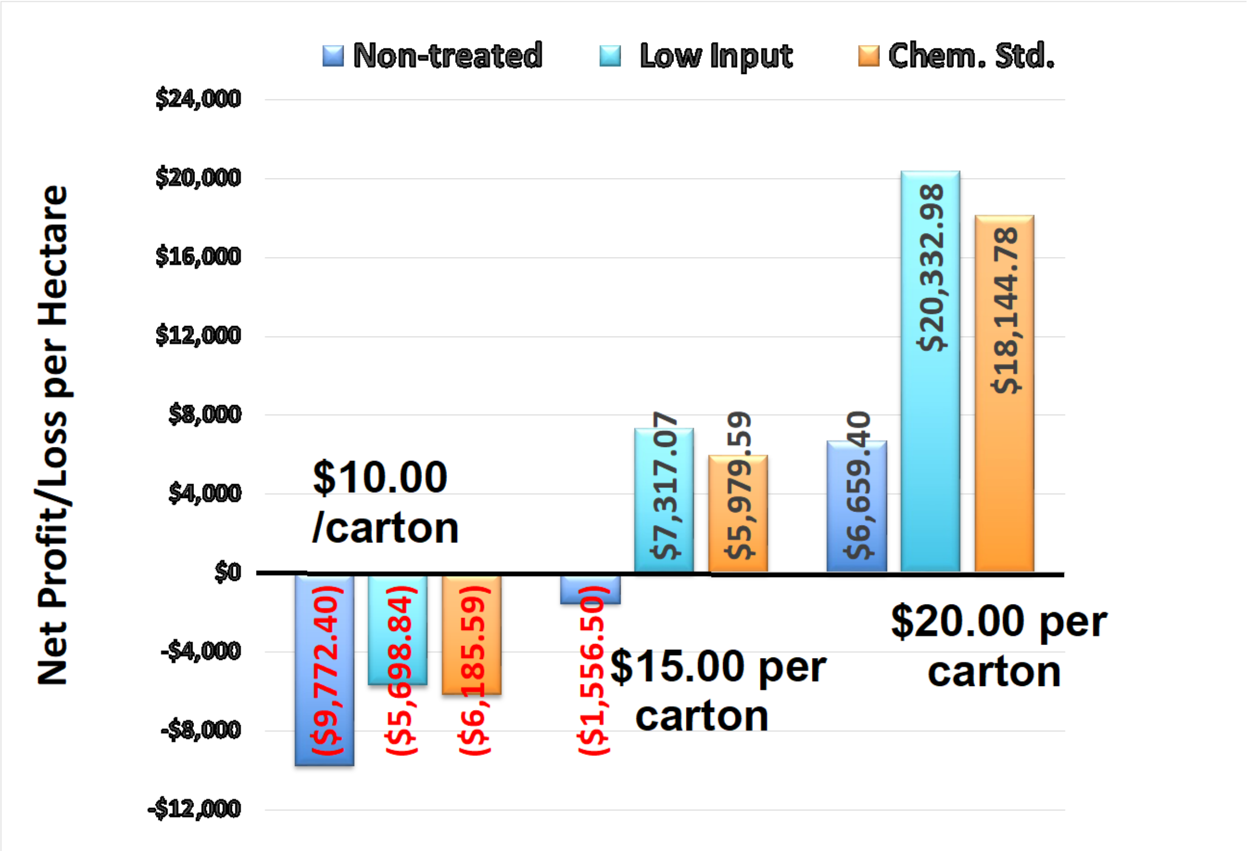
Objective II. Economic Analyses of an IPM and Chemical Standard Trial.

An economic analysis was prepared to compare harvest yields between treatments. Damage to the celery, costs of applications, and material costs were factored into the calculations. Figure 5 shows that the "Low Input" treatment performed the best at $15.00 and $20.00 per carton. The breakeven point for most growers is $10 to $12 per carton.

For this study, we tested new combinations of products designed to 1) reduce costs, 2) manage insecticide resistance, 3) increase harvest/yield, and 4) provide critical information to regulatory agencies on how products fit within an IPM program. The program consisted of a low input rotation of 1) Exirel®, Sequoia®, Radiant®, and Dipel® versus 2) Mustang Maxx®. In the IPM plots chemicals were applied 4 times over the course of an 18 week season. In the 'chemical standard' treatment the materials were applied 5 times.

We used very progressive chemical treatments with fewer applications for the low input program. In spite of using chemicals that target specific insects, which in most cases have higher costs per application, we were able to increase net profits for the Low Input IPM program. Other benefits such as environmental effects and worker health and safety aspects are improved in the IPM programs and are necessary to conform with the FQPA. As reported to the Cal EPA, without some pesticidal control, celery damage by insects will be extensive, and losses would not allow a reasonable profit to be made. Thus, some pesticides must continue to be registered for celery for the crop to remain viable in California.

Figure 5. Net profit or loss in IPM, chemical standard, and control treatments in 2023. Numbers associated with each bar are net profits (if numbers are red and in parentheses, they represent net losses).



Celery Residue Concerns

We continued to address the concerns of celery being classified in a consumer advocates publication as part of the dirty dozen regarding pesticide residues. The amount of pesticides used in crop protection in California declined from 1997-2009. Examination of the “Pesticide Use Report” (PUR) from 2022 (web: https://www.cdpr.ca.gov/) shows a trend for the use of more biopesticides, which are generally safer for the environment, workers, and consumers. The goal of current pest management programs is to reduce the amount of pesticide applied, which minimizes environmental, occupational, and consumer exposures.

Lygus Bug Resistance Research (A separate report was submitted)

We are continuing to develop quick and effective tools that can be used to determine levels of resistance of insects in field populations. Based on feedback from the Celery Research Advisory Board (CRAB), our initial efforts have been focused on the lygus bug. Lygus bug is also a pest for strawberry growers, and Dr. Perring had contacts within the Cal Poly Strawberry Center who were willing to assist us in our efforts to develop the biochemical tests necessary to detect resistance in field populations of lygus bugs.

Widespread resistance in several field collected populations in the Santa Maria, CA region have been detected. Pyrethroids are commonly used in many agricultural regions that celery is grown, and the next step will be to establish toxicity profiles for the susceptible lygus colony and other field collected populations. The recently appointed entomology program leader, Dr. Mohammad

Amir Aghae, of the Strawberry Center at Cal Poly San Luis Obispo has agreed to participate in this project. He has started sending field collected lygus bugs to our lab to establish lygus colonies for testing. These field collected colonies will be screened and compared to the susceptible lygus colony to determine the levels of pyrethroid resistance occurring in the field populations.

Success in this project will provide improved guidance toward making better decisions when choosing an insecticide.

Future Research

In our 2023 celery field trials we will be utilizing promising compounds that may be included in an IPM program. We will be testing IPM programs against a chemical standard program to continue to identify the most effective and economical pest control strategies. Some new insecticide materials are being developed in the industry and some are being tested in laboratory and IR-4 trials. As these products prove to be effective, we will continue to support the registration of these new products for celery use, which will continue to support the goals of the FQPA. Additionally, we will continue to pursue organic treatments that could fit into an IPM program for organic growers. We would like to test Aza-direct, Pure Crop1, Grandevo® and Venerate® in our organic IPM treatment rotation to determine if these products are viable options.

California Celery Research Advisory Board

Annual Project Report for 2023-2024

Insecticide Resistance of Celery Pests Focusing on the Lygus Bug, *Lygus hesperus*

Principal Investigator:	Dr. Chow-Yang Lee, Department of Entomology University of California, Riverside, CA 92521 chowyang.lee@ucr.edu
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Objectives:

1. Collect lygus bugs from various celery growing regions in the state and establish susceptible and resistant populations to a commonly used insecticide.
2. Develop biochemical assays to identify the resistance mechanisms in the resistant insect populations.
3. Develop PCR tests that will detect resistant genes within insect populations.
4. Develop a simple diagnostic field test method that will rapidly identify resistance.

Overview of work and procedures

Acquiring and rearing enough lygus bugs to do the resistance screening for the susceptible and field collected populations is critical for the completion of this project. Once we have collected and reared enough lygus bugs, the Lee lab will determine the insecticide resistance profiles, biochemical, and molecular diagnostic tools to determine resistance mechanisms in the populations.

Objective 1: Field collections and insecticide identification.

We will identify fields that have a history of problems managing lygus bugs and collect live insects for testing of potential resistance. Similarly, we will establish a susceptible lygus bug colony for comparison with the field collected populations. These insects will be used in the remaining objectives.

Objective 2: Develop biochemical assays.

The susceptible insect population will be used to establish a diagnostic concentration that will serve for insecticide resistance detection of the field population. Subsequent lab tests will be done to determine what rates kill the susceptible insect population. These baseline data of the susceptible population will be used for the determination of insecticide resistance ratios of the resistant insect populations. Biochemical tests using enzymatic assays will be used to screen insect populations. Four biochemical assays will be done: (1) total cytochrome P450, (2) elevated esterases, (3) glutathione S-transferase, and (4) altered acetylcholinesterase, using modified procedures of Lee et al. (2000) and Koou et al. (2014).

Objective 3: PCR tests
Target site mutations, namely kdr-resistance (conferring pyrethroid resistance) will be examined.

Objective 4: Diagnostic field test

A mortality-based simple vial diagnostic test will be developed for quick resistance detection in the field.

Results

Objective 1
Insects have been collected from commercial fields and will continue to be screened for levels of resistance using a common pyrethroid, bifenthrin. A susceptible lygus colony was acquired from the Strawberry Center at Cal Poly San Luis Obispo. The Strawberry Center project leader Dr. Mohammad Amir Aghaee has recently sent additional field collected *Lygus hesperus* bugs and they are now developing colonies.

Objective 2
The susceptible lygus colony was tested against bifenthrin to determine a baseline toxicological level. Five adult lygus bugs were placed into petri dishes with a 4 cm segment of green bean and replicated two times. The treated green beans were dipped into a solution of bifenthrin and allowed to air dry prior to the exposure (Figure 1). Mortality was recorded at 24 and 48 hours. The results are shown in Figure 3. These data will be used as a baseline to establish resistance ratios for the field collected populations. A field collected lygus bug colony, from an Agricultural Operations (AgOps) field at the University of California Riverside was tested for levels of resistance to bifenthrin. Table 1 specifies the location of the collection sites. Mortality at 24 and 48 hours for the AgOps lygus bugs is shown in Figure 4. Probit analyses of the susceptible and AgOps lygus colonies are shown in Table 2. The resistance ratios were calculated by dividing the LC_{50} value of each test population by the LC_{50} value of the susceptible population. The AgOps population showed 10X levels of resistance after 24 hours compared to the susceptible colony (2.04 ÷0.19=10.73). Additional field populations will be tested as they are acquired.

Figure 1. Dip treatment of green beans

Figure 2.

Figure 3. Percent mortality of the susceptible lab colony of *Lygus Hesperus*

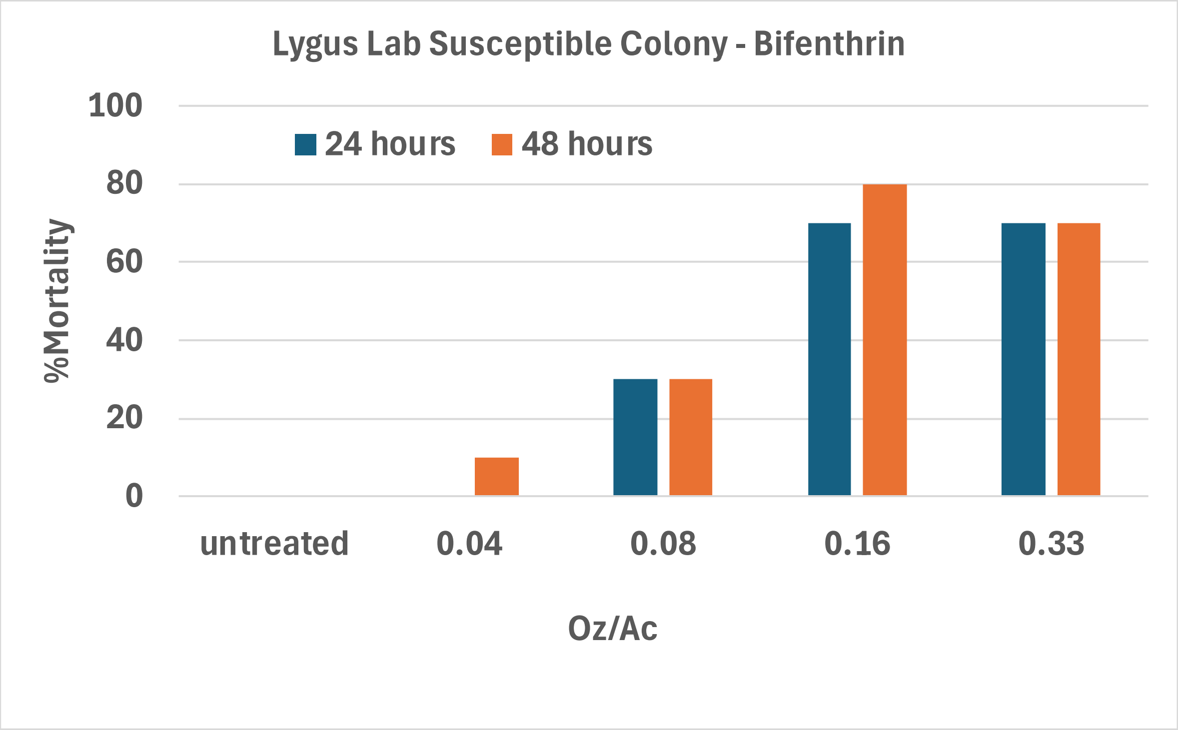

Figure 4. Percent mortality of AgOps field collected *Lygus Hesperus*

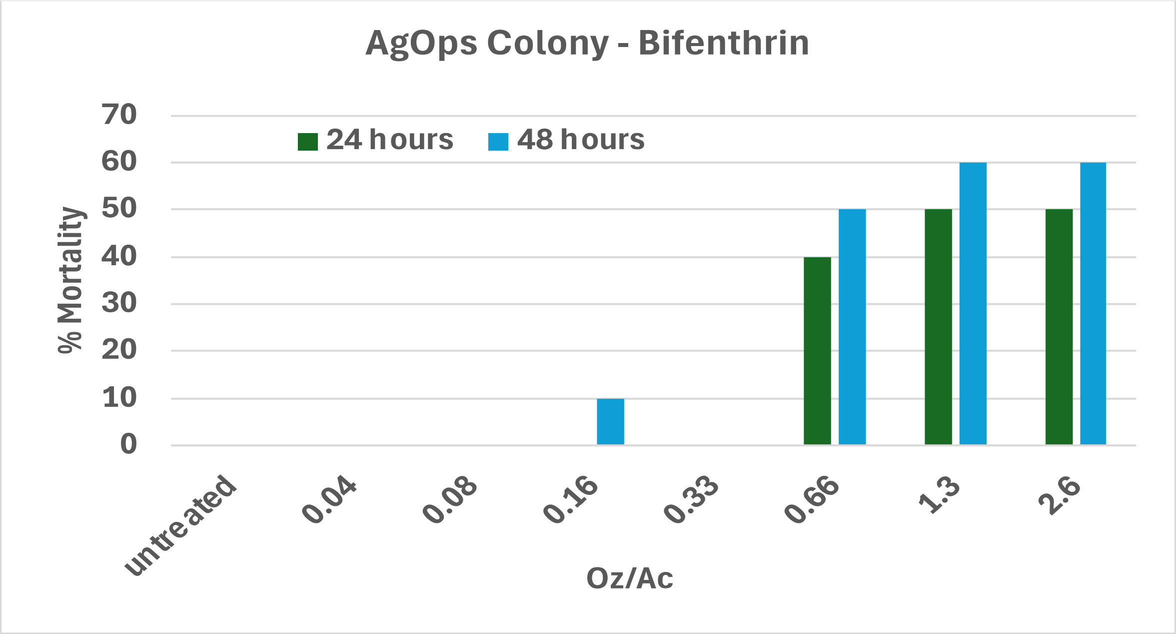

Table 1: Western tarnished plant bug(*Lygus hesperus*) test populations

Strain	Location	GPS	Collection Date
Susceptible	Lab susceptible, Arizona Univ., USA	n/a	Unknown
UCR AgOps	Riverside, CA, USA	33°57'54.5"N 117°20'25.5"W	6/20/2024

Table 2: Susceptibility (in ppm) of *Lygus hesperus* field populations relative to the laboratory susceptible strain.

Compound	Strain	n	LC_X	LC_X ± 95%CI		RR_{50}[a]	
				24h	48h	24h	48h
Bifenthrin (Brigade WSB)	Lab Susc.	50	50	0.19 (0.14-0.29)	0.18 (0.12-0.27)	-	-
			90	0.37 (0.28-0.62)	0.37 (0.27-0.64)		
			95	0.42 (0.32-0.72)	0.42 (0.31-0.75)		
			X^2	17.66	15.96		
			Slope	7.23	6.74		
	UCR AgOps	50	50	2.04 (1.54-3.14)	1.71 (1.28-2.54)	10.73	9.50
			90	3.60 (2.69-6.00)	3.20 (2.42-5.11)		
			95	4.04 (3.00-6.83)	3.62 (2.72-5.85)		
			X^2	18.70	21.75		
			Slope	0.82	0.86		

[a] The RR_{50} resistance ratio was calculated by dividing the LC_{50} value of each test population by the LC_{50} value of the susceptible population.

The next phase of this research as stated in the last section of "objective 2", after we have identified several resistant lygus populations, is to begin the biochemical analyses. These tests should provide answers regarding the mechanisms of resistance in the lygus bugs. Project objectives 3 and 4 will require additional funding in the future to attain the goal of having a field diagnostic test kit.