CALIFORNIA LEAFY GREENS RESEARCH PROGRAM
April 1, 2023 to March 31, 2024
REPORT

Project Title: USDA Lettuce Breeding for Improved Resistance to INSV, Pythium, Verticillium, and Fusarium

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ABSTRACT:
Lettuce production in California is significantly impacted by diseases including impatiens necrotic spot virus (INSV), Pythium Wilt, Verticillium Wilt, and Fusarium Wilt. Genetic resistance is the ideal, and often only, solution to control spread and reduce losses. For 2023-2024, our objectives were to: 1) develop lettuce germplasm resistant to INSV, 2) develop tools to evaluate germplasm resistance to Pythium wilt, 3) characterize resistance to Verticillium wilt races 1 and 2 and develop improved lettuce germplasm, and 4) characterize the Fusarium wilt variant isolate and develop germplasm with resistance. We validated field INSV results in the greenhouse and examined the roles virus localization, thrips counts, and total polyphenol content play in INSV resistance. We mapped the Eruption source of resistance to chromosome 2. We advanced germplasm in our INSV resistance breeding program. We optimized the Pythium inoculation protocol and conducted greenhouse trials to validate its efficacy to induce Pythium wilt infection. Final field and greenhouse evaluations were conducted on USDA breeding lines that combine Verticillium Race 1 and Race 2 resistance in preparation for germplasm release. We evaluated commercial and breeding germplasm for resistance to Fusarium race 1 and variant isolates in greenhouse and growth room experiments and selections were made for crossing. We identified a new variant Fusarium isolate (VSP-0916) that is more aggressive than race 1 and a potential threat to California production. We published papers on our research on INSV and Verticillium resistance breeding. All research efforts were to aid in the overall goal of releasing lettuce with improved resistance to diseases impacting production.

OBJECTIVES:
The long-range goal of this project is to release lettuce with improved resistance to diseases impacting production. Our objectives for 2023-2024 were to: 1) develop lettuce germplasm resistant to INSV, 2) develop tools to evaluate germplasm resistance to Pythium wilt, 3) characterize resistance to Verticillium wilt races 1 and 2 and develop improved lettuce germplasm, and 4) characterize the Fusarium wilt variant isolate and develop germplasm with resistance. From making initial crosses to releasing material- the germplasm, information, and tools developed and shared along the way are invaluable.

PROCEDURES:

Objective 1- Develop lettuce germplasm resistant to **impatiens necrotic spot virus (INSV)**.

Characterization of resistance to INSV and development of improved lettuce germplasm is imperative. Understanding the underlying mechanisms of resistance is critical for effective introgression and deployment in new varieties. For 2023-2024, we focused our INSV research efforts in three areas: 1. dissecting mechanisms of genetic resistance, 2. QTL mapping of the Eruption source of resistance, and 3. developing improved lettuce germplasm. Germplasm was evaluated in two replicated field experiments (planted in June and August 2023) to measure INSV incidence and severity under natural infection. Dr. Renee Eriksen and Dr. Daniel Hasegawa (USDA-ARS) collected samples to identify the roles virus localization, thrips counts, and total polyphenol content contribute to INSV resistance. We provided germplasm to Dr. Richard Michelmore (UC Davis) to identify lines that respond to his virally-encoded INSV proteins assay. Select breeding lines and the Batavia Reine des Glaces x Eruption RIL mapping population were evaluated in two greenhouse experiments using manual inoculation and viruliferous thrips transmission. INSV severity was measured weekly on a 0-5 scale and combined into Area Under the Disease Progress Stairs (AUDPS). QTL mapping was performed in collaboration with Dr. Ivan Simko (USDA-ARS) to locate the Eruption source of resistance. Breeding selections were made and advanced to the next generation in the greenhouse.

Objective 2- Develop a controlled-environment assay and evaluate germplasm resistance to **Pythium**. (sub-award to Dr. JP Dundore-Arias, CSUMB)

Pythium wilt continues to be a concern in the Salinas Valley and is responsible for significant lettuce yield losses. In 2023-2024, we collaborated with Dr. JP Dundore-Arias (CSUMB) to develop tools and evaluate varieties for Pythium resistance. A screening protocol was developed to assess lettuce germplasm tolerance/susceptibility to Pythium wilt. After the inoculation protocol was optimized, a trial was conducted twice to test the efficacy of the Pythium inoculum to induce Pythium wilt infection. Here, lettuce seedlings were transplanted into Pythium-infested soil, with non-inoculated plants serving as controls for comparison. Multiple replications (6) were included for each treatment to ensure reliable and accurate results. This protocol resembled the natural Pythium wilt infection process through infested soil, facilitating the evaluation of breeding lines varying in field susceptibility and/or tolerance to the disease.

Objective 3- Characterize resistance to **Verticillium wilt** Races 1 and 2 and develop improved lettuce germplasm.

Genetic resistance is the most economical and environmentally sound control method for Verticillium wilt. Complete resistance to race 1 is conferred by *Vr1*, but no germplasm has been identified that completely prevents infection by race 2. New isolates are being introduced on spinach seed, a common rotation crop with lettuce. Therefore, characterization of resistance to races 1 and 2 and development of improved lettuce germplasm is imperative. For 2023-2024, our work focused on three populations to introgress resistance to races 1 and 2 into improved lettuce germplasm for release. Germplasm segregating for *Vr1* was evaluated for resistance in race 1-infested field experiments and screened with the *Vr1*-linked molecular marker. All germplasm was evaluated for horticultural traits and severity of vascular discoloration in preparation for release.

Objective 4- Characterize the **Fusarium wilt** variant isolate and develop germplasm with resistance.

Fusarium wilt emerged as a major problem in the Salinas Valley in 2019. In recent years, there has been a significant increase in incidence and severity. Our research from 2022-2023 identified a potentially new race in coastal California, which could significantly impact lettuce production. For 2023-2024, we evaluated germplasm including popular varieties, released public material, and breeding lines to 1. identify lines resistant to both Fusarium race 1 and the variant isolate for breeding parent selection and 2. identify lines with variable reaction to race 1 and the variant isolate for addition to the differential set. Breeding lines were tested in the greenhouse under Fusarium race 1 and the variant isolate, and in the field (race 1) in Yuma, AZ in collaboration with Dr. Stephanie Slinski (Univ. Arizona).

RESULTS AND DISCUSSION:

Objective 1- Develop lettuce germplasm resistant to **impatiens necrotic spot virus (INSV)**.

In June 2023, seed of 162 recombinant inbred lines (RILs) of the Batavia Reine des Glaces x Eruption (BxE) mapping population, 57 potential parental lines, 29 entries from Enza Zaden, and 196 early generation USDA breeding lines was planted in a replicated experiment at Spence Farm under natural INSV infection. 10 plants per plot were evaluated weekly using a 0-5 INSV severity scale from 6 through 10 weeks after planting. Weekly data was combined into AUDPS to represent performance over the entire experiment. The natural INSV disease pressure in the field in 2023 was lower than in the past five years. The mean INSV incidence (percent of plants showing INSV symptoms) was less than 1% in the June 2023 planting. This is too low to make reliable evaluation of INSV resistance. Most lines had mean incidence of 0%, which falsely suggests resistance. Caution should be used when making selections under low disease pressure. Because the INSV pressure was so low, we took detailed notes on horticultural traits. This is not possible in years with typical disease pressure. With hopes that the INSV disease pressure would increase later in the season as expected in typical years, in August 2023, we planted seed of 162 RILs of the BxE mapping population, 96 plant introduction (PI) accessions from the National Plant Germplasm System (NPGS) collection, commercial varieties in collaboration with BASF/Nunhems, Nipomo Native Seed, Rijk Zwaan, Sakata, and Seminis, and 6 spinach varieties in collaboration with Dr. Jim Correll (Univ. Arkansas) in a replicated experiment at Spence Farm as described above. At three, six, and nine weeks after planting, plants were sampled from 8 select BxE RILs and parents (one plant per line per rep) for virus localization (by ELISA) and adult and immature thrips counts by Dr. Daniel Hasegawa and for total polyphenol content analysis by Dr. Renee Eriksen (Table 1). ELISA results were closely correlated to INSV severity, but thrips counts and total polyphenol content were not clearly correlated with INSV. Positive ELISA samples were identified in leaf, crown, and root tissues of all susceptible lines tested. In the resistant lines, of the 21 plants sampled only one had a barely-positive ELISA result in the leaf tissue (raw absorbance of 0.265). This suggests a mechanism of resistance in the Eruption source is the ability to prevent the virus from travelling systemically through the plant into the leaves. Interestingly, we saw positive ELISA samples at 9 weeks after planting, but no INSV symptoms were present then. It was only at 12 weeks after planting that symptoms arrived. This suggests that the virus is present in detectable levels in the field at least three weeks prior to symptoms appearing. Counts of adult and immature thrips do not appear to clearly correlate with ELISA or INSV severity data; the resistant RIL BxE16-017 had the highest number of adult

(41.33) and immature (47.67) thrips. Total polyphenol content also does not clearly correlate with ELISA or INSV severity data; the resistant RIL BxE16-024 (4219.84) and susceptible RIL BxE16-118 (4498.70) had similar results and values ranged widely across all lines tested.

Table 1. Plant sampling at 3, 6, and 9 weeks after planting of 8 selected BRG x Eruption mapping population RILs and parents for ELISA results (% INSV positive) from leaf, crown, and root tissue, INSV severity scores (0-5) at 9 and 12 weeks after planting, and count of adult and immature thrips and total polyphenol content at 9 weeks after planting.

		FIELD KRSP23-2 (Planted August 2023)							
		ELISA % Pos Wk9			SevWk9	SevWk12	Wk9 Thrips		Wk9 Total
Entry	Selection	Leaf (3)	Crown (3)	Root (3)	(10)	(10)	Adult	Immature	Polyphenols
BRG	SUSCEPTIBLE	100.0	66.7	100.0	0.40	1.93	17.00	17.00	226.94
Eruption	RESISTANT	0	33.3	0	0.00	0.03	12.33	28.67	786.79
BxE16-010	RESISTANT	0	0	0	0.00	0.63	20.33	39.67	872.01
BxE16-017	RESISTANT	0	0	0	0.00	0.37	41.33	47.67	1447.81
BxE16-024	RESISTANT	0	0	66.7	0.00	0.23	19.00	13.67	4219.84
BxE16-043	RESISTANT	0	0	0	0.00	0.10	12.00	21.67	405.31
BxE16-070	RESISTANT	0	33.3	0	0.00	0.13	24.33	7.67	1147.14
BxE16-118	SUSCEPTIBLE	100.0	100.0	100.0	0.13	1.63	22.67	32.67	4498.70
BxE16-140	SUSCEPTIBLE	66.7	100.0	100.0	0.00	1.53	14.67	23.00	239.09
BxE16-153	RESISTANT	33.3	66.7	100.0	0.00	0.33	12.67	31.33	10680.31

INSV symptoms only started appearing at 10 weeks after planting and INSV incidence data was recorded at 12 weeks after planting. Results were shared with industry collaborators with the reminder to use caution when making selections as the INSV pressure was extremely low in this trial (data not shown). The INSV pressure was still too low for reliable QTL mapping, so we instead took detailed notes on horticultural traits of the mapping population RILs. While the overall mean of INSV incidence was low amongst the NPGS accessions, variation was identified (Table 2). Of the entries tested, 83 accessions had incidence means not significantly different than the resistant check (Eruption) and 58 entries had INSV incidence below 50%. Caution must be used when making selections under low INSV pressure.

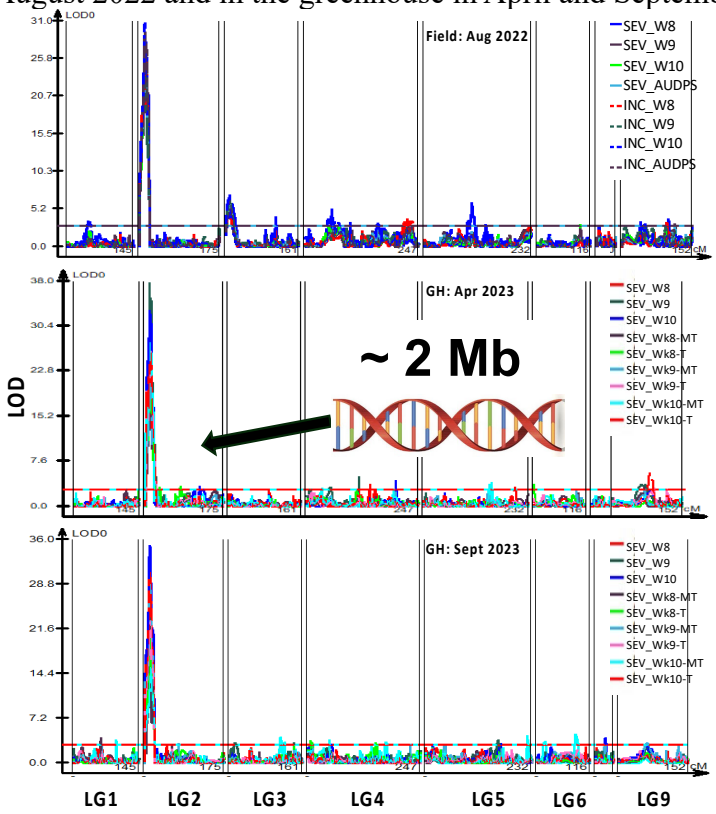
Table 2. Least square mean of INSV incidence (% symptomatic plants), standard error, and Tukey HSD results of Defender (susceptible check), Eruption (resistant check), and 96 plant introduction (PI) accessions from the National Plant Germplasm System collection in the August-planted 2023 INSV field trial.

Entry	LS Mean	Std Error	Tukey HSD		Entry	LS Mean	Std Error	Tukey HSD
Defender	64.29	9.32	ABCDEFGH		PI 491072	44.73	9.32	ABCDEFGH
Eruption	8.29	9.32	H		PI 491249	45.74	11.33	ABCDEFGH
PI 491111	7.21	15.92	ABCDEFGH		PI 204707	46.14	9.32	ABCDEFGH
PI 491134	16.19	9.32	GH		PI 184113	46.31	9.32	ABCDEFGH
PI 503633	16.61	9.32	GH		PI 176980	46.31	9.32	ABCDEFGH
PI 491061	17.23	9.32	FGH		PI 285651a	46.48	9.32	ABCDEFGH
PI 342519	18.43	9.32	EFGH		PI 491012	46.64	9.32	ABCDEFGH
PI 289040	20.05	9.32	DEFGH		PI 284702	47.36	9.32	ABCDEFGH
PI 491242	22.77	9.32	CDEFGH		PI 491086	48.42	9.32	ABCDEFGH
PI 204708	23.40	9.32	CDEFGH		PI 184786	49.37	9.32	ABCDEFGH
PI 491065	23.51	9.32	CDEFGH		PI 491062	49.61	9.32	ABCDEFGH

PI 342442	24.27	9.32	CDEFGH		PI 261651	50.12	9.32	ABCDEFGH
PI 288244b	25.00	9.32	CDEFGH		PI 491079	50.35	9.32	ABCDEFGH
PI 379362	25.15	9.32	CDEFGH		PI 190906	50.78	9.32	ABCDEFGH
PI 273597	25.36	9.32	CDEFGH		PI 491078	51.35	9.32	ABCDEFGH
PI 491064	27.11	9.32	BCDEFGH		PI 120962	52.16	11.33	ABCDEFGH
PI 503605	27.75	9.32	BCDEFGH		PI 187238	52.26	9.32	ABCDEFGH
PI 491230	28.39	9.32	ABCDEFGH		PI 491011	52.31	9.32	ABCDEFGH
PI 491023	28.69	9.32	ABCDEFGH		PI 269498	52.38	9.32	ABCDEFGH
PI 342441	29.26	9.32	ABCDEFGH		PI 491085	52.65	9.32	ABCDEFGH
PI 503640	31.39	9.32	ABCDEFGH		PI 491063	52.78	9.32	ABCDEFGH
PI 503607	31.47	9.32	ABCDEFGH		PI 381932	54.24	9.32	ABCDEFGH
PI 206965	31.63	9.32	ABCDEFGH		PI 491068	54.34	9.32	ABCDEFGH
PI 491140	33.32	9.32	ABCDEFGH		PI 273431	55.32	9.32	ABCDEFGH
PI 491110	33.36	11.33	ABCDEFGH		PI 184113a	55.71	9.32	ABCDEFGH
PI 491059	33.37	9.32	ABCDEFGH		PI 491067	56.19	9.32	ABCDEFGH
PI 491245	33.94	15.92	ABCDEFGH		PI 289032a	56.74	9.32	ABCDEFGH
PI 379356	34.09	15.92	ABCDEFGH		PI 202349	56.81	9.32	ABCDEFGH
PI 491024	35.80	9.32	ABCDEFGH		PI 182208B	57.50	9.32	ABCDEFGH
PI 250428	36.29	9.32	ABCDEFGH		PI 288244	57.53	9.32	ABCDEFGH
PI 491100	37.58	9.32	ABCDEFGH		PI 273594	57.73	9.32	ABCDEFGH
PI 142871a	38.54	9.32	ABCDEFGH		PI 249536	59.19	9.32	ABCDEFGH
PI 289037a	39.08	9.32	ABCDEFGH		PI 273596	60.45	9.32	ABCDEFGH
PI 491077	39.11	9.32	ABCDEFGH		PI 491232	60.79	9.32	ABCDEFGH
PI 289061	39.72	9.32	ABCDEFGH		PI 198733	61.25	9.32	ABCDEFGH
PI 491122	40.04	9.32	ABCDEFGH		PI 491025	64.29	9.32	ABCDEFGH
PI 323940	40.36	9.32	ABCDEFGH		PI 491074	66.69	9.32	ABCDEFG
PI 491009	40.84	9.32	ABCDEFGH		PI 269500	67.01	9.32	ABCDEFG
PI 491104	41.15	9.32	ABCDEFGH		PI 288244a	67.78	9.32	ABCDEFG
PI 184789	41.73	9.32	ABCDEFGH		PI 491210	69.97	9.32	ABCDEFG
PI 491107	41.94	9.32	ABCDEFGH		PI 184788	71.50	9.32	ABCDEFG
PI 274366	41.98	9.32	ABCDEFGH		PI 491070	73.14	9.32	ABCDEF
PI 491080	42.02	9.32	ABCDEFGH		PI 323940a	74.13	9.32	ABCDE
PI 140394	42.93	9.32	ABCDEFGH		PI 211600	75.35	9.32	ABCD
PI 390975	43.54	9.32	ABCDEFGH		PI 183234	76.26	9.32	ABC
PI 342552	43.75	15.92	ABCDEFGH		PI 491007	76.26	9.32	ABC
PI 491071	44.07	9.32	ABCDEFGH		PI 491008	76.38	9.32	ABC
PI 274358	44.38	9.32	ABCDEFGH		PI 250020	81.79	9.32	AB
PI 271475	44.71	9.32	ABCDEFGH		PI 491020	84.44	9.32	A

In April and September 2023, we planted seed of 162 RILs of the BxE mapping population in two replicated experiments in our INSV greenhouse under infection by manual inoculation and viruliferous thrips transmission to validate August 2022 field results. In each of three replications, one plant per line was infected by both manual inoculation and viruliferous thrips transmission and one plant per line was infected by viruliferous thrips transmission only. Each plant was evaluated weekly using a 0-5 INSV severity scale from 6 through 10 weeks after planting. QTL mapping was run on August 2022 field data and April and September 2023 greenhouse data sets and the same QTL was identified in each experiment (Figure 1). We are moving forward with fine mapping this region in collaboration with Enza Zaden.

Figure 1. QTL mapping of the Eruption source of INSV resistance in 162 recombinant inbred lines from the Batavia Reine des Glaces x Eruption mapping population evaluated in the field in August 2022 and in the greenhouse in April and September 2023.



In December 2023, we planted seeds of 23 lines from BASF/Nunhems (under an MTA), 65 selected NPGS accessions, 8 Fusarium wilt differential varieties, 2 commercial varieties suggested by Dr. Yu-Chen Wang (UCCE), and checks in a replicated experiment in our INSV greenhouse under infection by manual inoculation and viruliferous thrips transmission as described above. The majority of entries were not significantly different from the susceptible check (Table 3). Only two accessions, PI 273597 and PI 491065, were significantly lower than the susceptible check and not significantly different from the resistant check.

In 2024, we published the paper "Evaluation of lettuce germplasm for resistance to impatiens necrotic spot virus" in the journal Euphytica with Dr. Santosh Nayak, Dr. Daniel Hasegawa, and Dr. Renee Eriksen detailing the dissection of mechanisms of resistance as detailed in our 2022-2023 annual report (details in References section).

Table 3. Least square mean of INSV severity (0-5) at 10 weeks after planting and Tukey HSD results of Defender (susceptible check), Eruption (resistant check), and plant introduction (PI) accessions in a greenhouse experiment under manual inoculation and viruliferous thrips inoculation.

Entry	LSMean	Tukey HSD		Entry	LSMean	Tukey HSD
Defender	5.00	A		PI 503605	3.83	ABC
PI 491011	5.00	A		PI 142871a	3.67	ABC
PI 491020	5.00	A		PI 176980	3.67	ABC

PI 491023	5.00	A		PI 274358	3.67	ABC
PI 491230	5.00	A		PI 274366	3.67	ABC
PI 285651a	4.83	AB		PI 491059	3.67	ABC
PI 491012	4.83	AB		PI 491100	3.67	ABC
PI 289061	4.67	AB		PI 491104	3.67	ABC
PI 491072	4.67	AB		PI 491107	3.67	ABC
PI 491077	4.67	AB		PI 503633	3.67	ABC
PI 491140	4.67	AB		PI 249536	3.50	ABC
PI 491250	4.67	AB		PI 271475	3.50	ABC
PI 491068	4.50	AB		PI 323940	3.50	ABC
PI 269500	4.50	AB		PI 342442	3.50	ABC
PI 204708	4.33	AB		PI 491122	3.50	ABC
PI 288244	4.33	AB		PI 491086	3.33	ABC
PI 491024	4.33	AB		PI 503607	3.33	ABC
PI 491061	4.33	AB		PI 184789	3.17	ABC
PI 491249	4.33	AB		PI 381932	3.17	ABC
PI 288244b	4.33	AB		PI 503640	3.00	ABC
PI 184113	4.17	AB		PI 491242	3.00	ABC
PI 250428	4.17	AB		PI 491110	3.00	ABC
PI 390975	4.17	AB		PI 342552	3.00	ABC
PI 491064	4.17	AB		PI 342519	3.00	ABC
PI 140394	4.00	ABC		PI 491111	2.67	ABC
PI 206965	4.00	ABC		PI 491134	2.67	ABC
PI 491080	4.00	ABC		PI 342441	2.67	ABC
PI 491085	4.00	ABC		PI 379362	2.67	ABC
PI 204707	3.83	ABC		PI 289037a	2.50	ABC
PI 211600	3.83	ABC		PI 491071	2.50	ABC
PI 289040	3.83	ABC		Eruption	1.83	BC
PI 491009	3.83	ABC		PI 491065	1.83	BC
PI 491245	3.83	ABC		PI 273597	1.00	C

Objective 2- Develop a controlled-environment assay and evaluate germplasm resistance to **Pythium**. (sub-award to Dr. JP Dundore-Arias, CSUMB)

A Pythium inoculation protocol was developed (Figure 2) following previously published methods with corresponding modifications (Quesada-Ocampo, et al., 2009; Quesada-Ocampo, et al., 2016). Inoculations involved hulled millet grains inoculated with previously isolated and purified *Pythium uncinulatum* isolates. Hulled millet grains (160g) were mixed with water (120mL), L-asparagine (0.128g), calcium carbonate (4g), and calcium sulfate (gypsum) (4g) in glass Erlenmeyer flasks (1000mL), covered with aluminum foil. After mixing, the millet media was autoclaved twice. Approximately five plugs, each with a diameter of 1 cm, from *P. uncinulatum* isolates previously cultured for 10-12 days on corn meal agar (CMA), were introduced into a sterile millet medium. For the mock-inoculated control, plugs of CMA without *P. uncinulatum* were used (Figure 2). The inoculated flasks were subsequently stored in a dark environment at room temperature (21-23 C). Approximately 3-4 days after incubation, when a third of the millet grains showed white mycelia, flasks were manually shaken to disperse the pathogen and expedite the medium colonization. The entire millet medium within a flask was typically fully colonized around 20 days after inoculation (Figure 3). At that point, inoculum (1g) was plated on CMA to confirm growth morphology consistent with *P. uncinulatum* and discard any potential contamination. The prepared millet inoculum was mixed with soil or potting

substrate in a 1:7 ratio (Figure 4). Lettuce transplants of approximately three weeks were planted directly into the inoculated substrate. After three weeks of growth, plants were harvested, and roots were washed to remove debris. Symptomatic roots were observed under a microscope for the presence of characteristic *P. uncinulatum* oospores, and a subset of roots were surface sterilized before plating on CMA for confirmation.

The protocol was validated with three lettuce varieties (Duquesne, Telluride, and Copious) in two replicated trials. Each trial included planting each variety in inoculated and non-inoculated substrate, with six plants per treatment. Plants in the inoculated and non-inoculated treatments were evaluated for total, shoot, and root biomass, as well as root length (Figure 5). Inoculating with Pythium-infested millet consistently affected plant growth, including both below and aboveground development (Figure 6). Commercial varieties exhibited distinct responses to Pythium wilt infection. Generally, Duquesne and Telluride showed the greatest growth inhibition across all parameters, while Copious appeared to be the least affected. Telluride and Copious showed lower inhibition of root length, primarily reflecting elongation of the taproot. The variability in root biomass versus root length suggests that Pythium infection, under the tested conditions, might affect lateral/feeder root development more than the taproot. These findings are consistent with field observations where symptomatic roots of severely infected plants exhibit a 'rat tail' appearance, characterized by the lack of lateral roots. These findings indicate differing levels of susceptibility to Pythium wilt among cultivars, underscoring the importance of selecting varieties with enhanced resistance for improved performance in naturally infested soils.

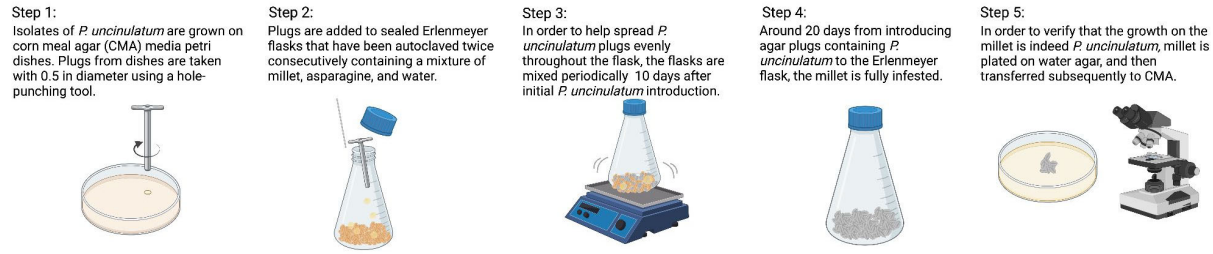


Figure 2. Inoculum preparation protocol.

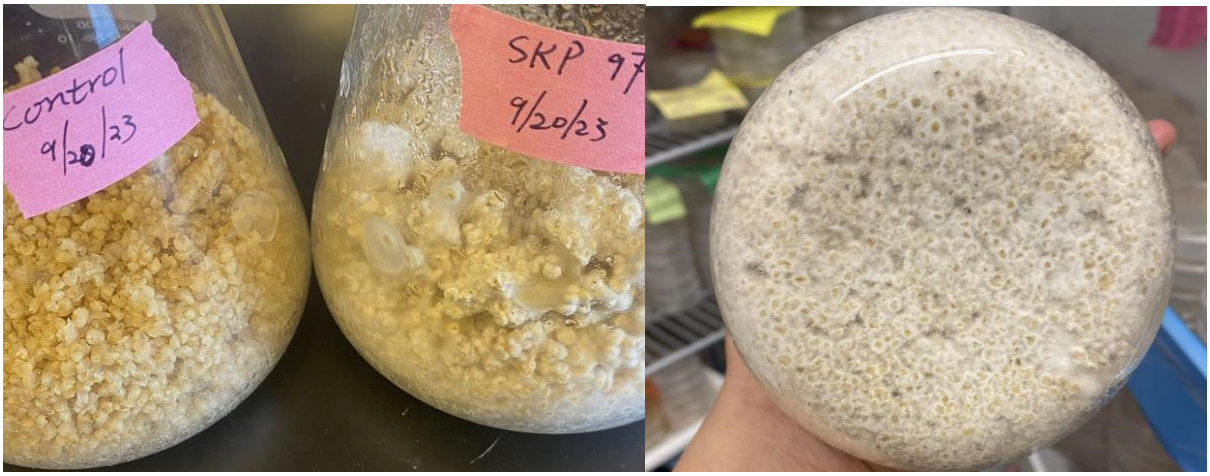


Figure 3. Millet inoculum. Side-by-side mock-inoculated control and *P. uncinulatum*-inoculated millet grain (Left). In contrast with control millet grains, abundant mycelium is observed in inoculated grains (Right).

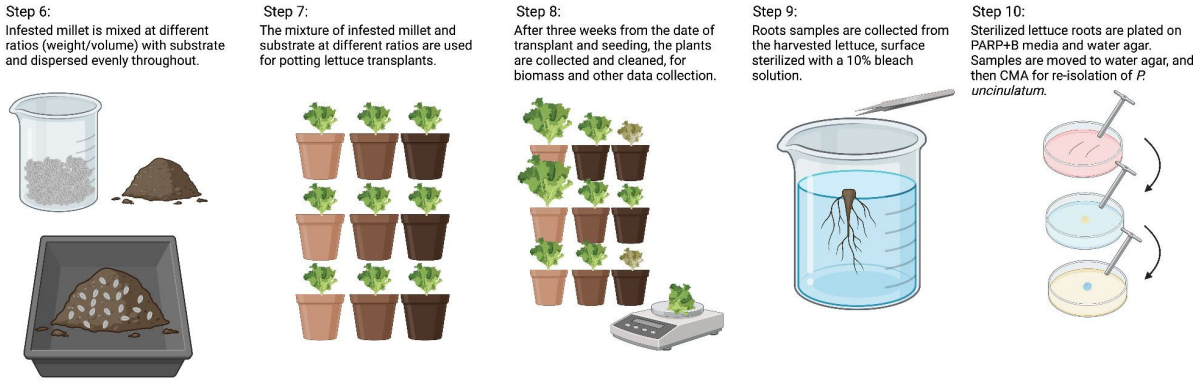


Figure 4. Soil infestation protocol.

Figure 5. Visual examination of plant biomass between Duquesne plants grown in control (left) and inoculated substrate (right).

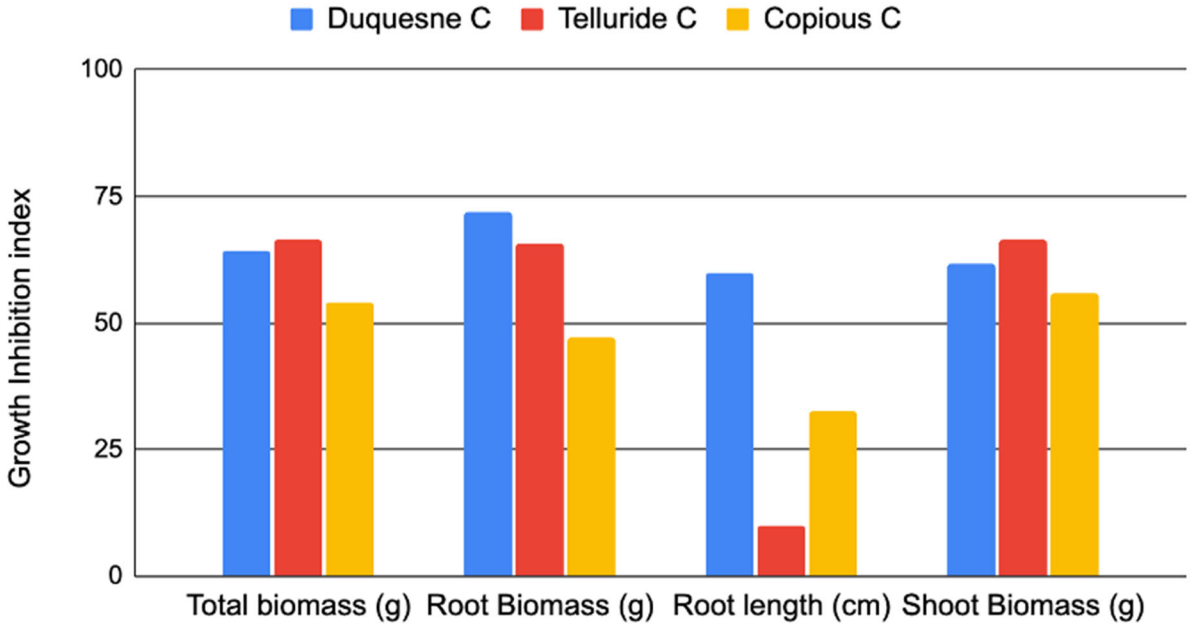


Figure 6. Comparative growth inhibition index across three cultivars (Duquesne, Telluride, and Copious) for different biomass parameters. Each bar represents the calculated growth inhibition

index, expressing the extent to which growth was hindered in response to *P. uncinulatum* inoculation, with values ranging from 0 (no inhibition) to 100 (complete inhibition).

Objective 3- Characterize resistance to **Verticillium wilt** Races 1 and 2 and develop improved lettuce germplasm.

In June 2023, seed of 66 USDA breeding lines with resistance to Verticillium races 1 and 2, 19 parental lines, 4 Fusarium differential varieties, and 7 lines from Salinas Valley Seeds (under MTA) was planted in a replicated experiment in Field C on the USDA station. Field C was fumigated in 2018 and in 2019 and 2020 was transplanted with over 20,000 Verticillium race 1-inoculated seedlings. Field trials from 2020-2022 resulted in successful evaluation for Verticillium race 1 resistance, but Fusarium root symptoms were significant in 2023 tests. To account for this change, both Verticillium root symptom severity (on a 0-5 scale) and Fusarium root symptom severity (rated 0, 1, 3, or 5) were recorded on 10 plants per plot at 12 weeks after planting (Table 4). 23% of sampled plants had moderate to severe Verticillium symptoms and 28% had moderate to severe Fusarium symptoms. 26 USDA breeding lines were selected for low Verticillium and Fusarium root symptoms and advanced to the next generation in the greenhouse. Fusarium seems to be increasing in severity throughout Field C. Dr. Nick LeBlanc identified both verticillium and fusarium in plant and soil samples collected in 2023. While we can no longer use this space for evaluating material for Verticillium race 1 resistance only, it is an ideal location to evaluate lines under both Verticillium race 1 and Fusarium race 1 at the same time. It is an objective of our breeding program to release germplasm with resistance to multiple diseases and this field will support that aim.

In 2023, we published the paper “Inheritance of partial resistance to isolate VdLs17 of *Verticillium dahlia* within *Lactuca* spp.” in the journal Plant Disease with Dr. Santosh Nayak detailing the inheritance of resistance to race 2 from two sources as detailed in our 2021-2022 annual report (details in References section).

Table 4. Verticillium root symptom severity (0-5) and Fusarium room symptom severity (0,1,3,5) of entries tested in the June 2023 planted field trial under Verticillium race 1-inoculated conditions. Selected lines were advanced in the breeding program.

Entry	Selected	Gen	Verticillium (0-5)	Fusarium (0,1,3,5)
22-101 (Primo)			1.33	4
22-102			1.53	3.1
22-103			0.63	0.73
22-104			0.27	0.53
22-105 (San Miguel)			0	0.23
22-106			0.43	0.5
22-107			0.87	2.37
Costa Rica No. 4			0.33	0.57
Banchu Red Fire			1.03	2.4
Patriot			0.97	2.77
Romabella			0.6	0
Amazona			0.07	0.23
Anais			0.33	3.83
Ancora			0.93	0.73
Blas			1.13	1.43
Calmer			1.53	2.1
Conquistador			0.3	0.13
El Guapo			1.83	3.36
Eruption			0.2	0.27
Grizzly			1.35	3.53
La Brillante			0.53	2.1
PI 171674			0.23	0.2
Reine des Glaces			0.2	0.5
RH12-3196			0.87	2.97
Salinas			1.43	1.4
Salinas 88			1.77	1.17
San Andreas			0.13	0.63
Sentry			0	0.43
Tamarack			1.38	3.05
Vanguard			1.22	1.99
KR21-0521		F3	0.73	1.2
KR21-0537		F3	0.47	2.43
KR21-0542		F3	1.57	2.1
KR21-0723	x	F3	0.23	0.73
KR21-0735	x	F3	0.43	0.63
KR21-0984		F3		
KR21-0991	x	F3	1.47	3.63
KR22-0090		F3	1.2	1.63
KR22-0096		F3	0.67	1.23
KR22-0097		F3	0.7	1.63
KR22-0112	x	F3	0	0.03
KR22-0122	x	F3	0.2	0.4
KR22-0148		F3	0.83	2.01
KR22-0163	x	F3	1.3	3
KR22-0217		F4	0.3	1.6
KR22-0218		F4	0.5	1.7
KR22-0219		F4	0.48	2.23
KR22-0221		F4	0.73	2.47

Entry	Selected	Gen	Verticillium (0-5)	Fusarium (0,1,3,5)
KR22-0223		F4	1.13	2.37
KR22-0224		F4	0.77	2.33
KR22-0225		F4	0.47	2.57
KR22-0226		F4	0.77	2.7
KR22-0227		F4	0.57	1.53
KR22-0228		F4	0.63	2.07
KR22-0244		F4	0.4	1.67
KR22-0254		F4	1.07	1.57
KR22-0255		F4	1.1	1.9
KR22-0257		F4	0.87	2.58
KR22-0262		F4	1.23	1.67
KR22-0263		F4	1.03	1.27
KR22-0284		F4	1.17	1.67
KR22-0397		F4	0.23	1.43
KR22-0399	x	F4	0.07	0.23
KR22-0400	x	F4	0.2	1
KR22-0403	x	F4	0.17	1.07
KR22-0406		F4	0.47	1.7
KR22-0414		F4	1.3	2.5
KR22-0416	x	F4	0.43	0.47
KR22-0422	x	F4	0.4	1.03
KR22-0425		F4	0.73	2.93
KR22-0429	x	F4	0.27	0.3
KR22-0469	x	F4	0.47	1.07
KR22-0470	x	F4	0.5	0.37
KR22-0472	x	F4	0.1	0.6
KR22-0473	x	F4	0.43	0.43
KR22-0474	x	F4	0.2	0.53
KR22-0476	x	F4	0.27	0.07
KR22-0477		F4	0.67	1.57
KR22-0478	x	F4	0.6	0.97
KR22-0479	x	F4	0.07	0.2
KR22-0480	x	F4	0.2	0.33
KR22-0495	x	F4	0.17	0.57
KR22-0896		F4	0.7	1.63
KR22-1010		F4	0.63	0.8
KR22-1015	x	F4	0.1	0.17
KR22-1017		F4	0.63	1.07
KR22-1019	x	F4	0.2	0.07
KR22-1021	x	F4	0.5	0.07
KR22-1025		F4	0.73	2.13
KR22-1032		F4	0.67	1.07
KR22-1036	x	F4	0.1	0.51
KR22-1040		F4	0.53	2.13
KR22-1045		F4	0.7	2.1
KR22-1046		F4	0.63	2.2
KR22-1051		F4	1.43	1.8
KR22-1053		F4	0.87	1.9

Objective 4- Characterize the **Fusarium wilt** variant isolate and develop germplasm with resistance.

Since we confirmed the presence of a variant isolate of Fusarium wilt in California fields in 2022-2023, our Fusarium research efforts have shifted to also include germplasm evaluation in the greenhouse and growth room under Fusarium race 1 and the variant isolate. In May and July 2023, we tested 4 Fusarium differential varieties, 2 commercial varieties from Salinas Valley Seeds (under MTA), and parental lines in two greenhouse experiments under root dip inoculation with two variant isolates (VSP-0916 and Fol621s) and race 1. At 4 weeks after planting, plants were rated for Fusarium symptoms on a 1-4 scale (Table 5). VSP-0916 (received in collaboration with Dr. Alex Putman, UC Riverside) is of particular concern as it caused significant disease on

Costa Rica No. 4, a typically resistant differential variety. Results suggest VSP-0916 is more aggressive than race 1. Dr. Frank Martin (USDA-ARS) ran molecular diagnostic assays on race 1, VSP-0916, and Fol621s and the results were identical. Given the plant responses are so different amongst these three isolates, it suggests the currently available molecular diagnostic tools are not able to differentiate race 1 and variant Fusarium isolates. It is imperative to evaluate additional material for resistance to VSP-0916 and develop reliable molecular diagnostic tools.

Table 5. Least squares (LS) means of disease severity observed in lettuce cultivars against different isolates of *Fusarium oxysporum* f. sp. *lactucae*.

	VSP-0916		Fol621s		Race 1	
Entry	GH May23	GH Jul23	GH May23	GH Jul23	GH May23	GH Jul23
Patriot	4	4	3.9	4	4	4
Romabella	1	1	1	1.1	1	1.1
Banchu Red Fire	4	2	1.1	2.8	3.3	2.8
Costa Rica No. 4	3.8	4	1	1.1	1	1
22-101 (Primo)	4	4	1.8	1.4	2.3	3.9
22-105 (San Miguel)	2.1	1.9	4	4	4	4
Grizzly	4	4	3	3.9	4	4
Reine des Glaces	3.3	3.6	1	1	1	1.3
Eruption	4	3.1	2	2.6	3.8	4
$HSD_{0.05}$	1	1.2	1.3	0.9	0.9	0.4

In August 2023, we evaluated 6 USDA released Fusarium breeding lines (in collaboration with Dr. Jim McCreight (USDA-ARS), parental lines, and checks under six isolates of Fusarium including the aggressive VSP-0916. Breeding lines 15632, 15633, 15669, and 15670 appear to be good candidates for broad-spectrum Fusarium resistance (Figure 7). Select lines were evaluated in a Fusarium field trial in Fall 2023 in Yuma in collaboration with Dr. Stephanie Slinski (Univ. Arizona) and advanced in the breeding program.

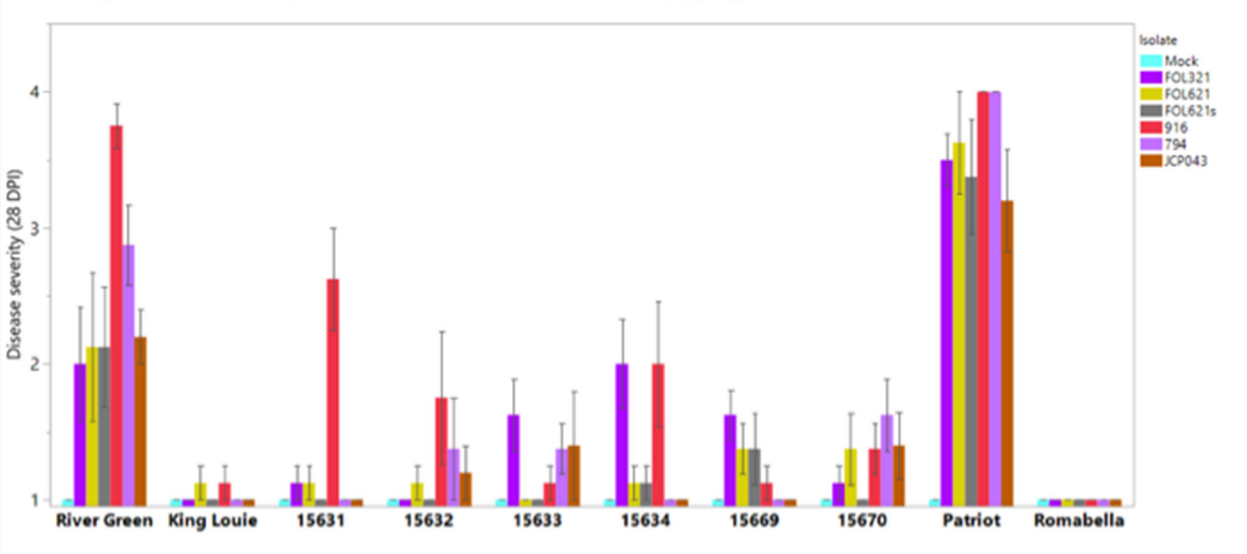


Figure 7. Least squares (LS) means of disease severity observed in USDA breeding lines against different isolates of *Fusarium oxysporum* f. sp. *lactucae*.

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