CALIFORNIA LEAFY GREENS RESEARCH PROGRAM
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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 2022-2023, our objectives were to: 1) develop lettuce germplasm resistant to INSV, 2) develop tools to evaluate germplasm resistance to Pythium, 3) characterize resistance to Verticillium wilt Races 1 and 2 and develop improved lettuce germplasm, and 4) develop germplasm with combined resistance to Fusarium wilt and Verticillium wilt. We validated field INSV results in the greenhouse and dissected the mechanisms of resistance among our most resistant germplasm with thrips-only and virus-only experiments. We selected 16 USDA breeding lines for advancement in our breeding program. We successfully validated field Pythium results using an *in-vitro* assay and made strides in developing a greenhouse Pythium evaluation protocol. 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. USDA breeding lines to combine Fusarium and Verticillium wilt resistances were evaluated in field and greenhouse experiments and selections made for germplasm release. 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 2022-2023 were to: 1) develop lettuce germplasm resistant to impatiens necrotic spot virus, 2) develop a controlled-environment assay to evaluate germplasm resistance to Pythium, 3) characterize resistance to Verticillium wilt Races 1 and 2 and develop improved lettuce germplasm, and 4) develop germplasm with combined resistance to Fusarium wilt and Verticillium wilt. 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).

In recent years, incidence of impatiens necrotic spot virus (INSV) has increased significantly causing devastating losses to lettuce production. Genetic resistance is the most economical and environmentally sound control method, but varieties that completely prevent infection are not available. Therefore, characterization of resistance to INSV and development of improved lettuce germplasm is imperative. For 2022-2023, we focused our research efforts in four areas: germplasm evaluation 1. in the field with natural INSV pressure, 2. in the greenhouse with manual inoculation and viruliferous thrips transmission, 3. in the greenhouse under thrips pressure with no virus, and 4. in the growth chamber under virus pressure with no thrips. Dissecting the underlying mechanism will direct characterizing the resistance and deploying it in commercial varieties. Germplasm was evaluated in two replicated field experiments (planted in June and August 2022) to measure INSV incidence and severity under natural infection. Results were confirmed 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). From previous field and greenhouse experiments, 13 lines were identified that had consistent resistant, intermediate, or susceptible INSV reactions. Dr. Daniel Hasegawa (USDA-ARS, Salinas) tested this germplasm under thrips pressure (no virus) in two sets of experiments under controlled greenhouse conditions: choice tests and no-choice tests. In choice tests, thrips had the ability to choose the host plant that it feeds on. 200 adult thrips were introduced into thrips-proof cages containing all 13 lines (one plant each). After 3 weeks, all plants were removed and counted for the number of thrips present on each plant (immature and adult). Feeding damage was assessed on the leaves. In no-choice tests, thrips were forced to feed on a provided food source, consisting of leaf samples, taken from the 13 lines at the 5-6 true leaf stage. For each line, a leaf sample was placed into a Munger cell containing 10 adult thrips and allowed to feed for 3 days. At the end of the experiment, Munger cells were disassembled and counted for the number of living thrips and scored for feeding damage. To help differentiate virus versus thrips resistance, we paired the thrips preference tests with a manual INSV inoculation only (no thrips pressure) of the same material. Five plants each of the 13 lines were grown in a thrips-free growth chamber. At the 4 true leaf stage, we manually inoculated all leaves of each plant with INSV sap (infected tissue in phosphate buffer). Plants were rated weekly for 5 weeks for INSV symptom severity on a scale from 0-5 and combined into AUDPS. At 5 weeks after inoculation, we harvested tissue from each plant to test for the presence of INSV using ELISA.

Objective 2- Develop a controlled-environment assay to evaluate germplasm resistance to Pythium.

Pythium wilt is becoming an increasing concern in the Salinas Valley and has been responsible for significant lettuce yield losses. Previous work suggests Pythium tolerance already exists to various degrees within commercial varieties. Tools are needed to quickly evaluate varieties for resistance. Dr. JP Dundore-Arias (CSU-Monterey Bay) evaluated tolerance to Pythium wilt among seventeen commercial lettuce varieties using an *in-vitro* assay (Figure 1).

Figure 1. Illustration of Pythium wilt tolerance *in-vitro* assay. Top images correspond to mock-inoculated plants, while bottom images represent *P. uncinulatum*-inoculated plants. Left: arrangement seedling in disinfested polypropylene boxes. Center: close up of root discoloration symptoms. Right: Root tissue evaluated under the microscope for presence of oospores.

These varieties represent a subset of a larger number (n=53) previously tested in a field trial for their field tolerance to the disease. Briefly, 1-mm diameter agar plugs of two previously sequenced pathogenic *Pythium uncinulatum* isolates growing on selective media were placed in contact with previously germinated lettuce seedlings on autoclaved filter paper in disinfested polypropylene boxes and maintained at room temperature. Symptom development and mortality were evaluated for 120-h counting the number of seedlings showing root discoloration and necrosis and comparing them to the mock-inoculated control consisting of Pythium-free agar plugs. Each variety was replicated three times testing a total of 72 inoculated seedlings for each variety. Additional work was conducted by Dr. Dundore-Arias to test the efficacy of different inoculation methods with the goal of implementing a complementary *in-vivo* screening method to further evaluate germplasm for Pythium wilt tolerance. Four inoculation methods commonly used in the study of other Pythium root rot diseases were tested (Figure 2), including 1) root dipping, 2) soil drench, 3) addition of the pathogen in agar plugs, and 4) an oat-V8-vermiculite inoculum as previously described with modifications.

Method 1: Dip. Exposed seedlings roots placed in a petri dish containing a blended slurry of three different *Pythium uncinulatum* isolates in sterile water.

Method 2: Drench. Seedlings were transplanted and drenched with 30 mL of a blended slurry of three different *Pythium uncinulatum* isolates in sterile water.

Method 3: Agar plug. Transplanted seedlings were inoculated with three 5-mm agar plugs of different isolates at the base of the seedling and secured with parafilm.

Method 4: Vermiculite. Isolates were grown in a mixture of sterile vermiculite, oats, and clarified V8 juice for 4-weeks and mixed into soil.

Figure 2. Illustration of Pythium wilt inoculation methods.

In the root dip inoculation method, lettuce seedlings were inoculated by placing them in a petri dish containing a blended slurry of *P. uncinulatum* isolates in sterile water and letting them soak for 15-min (Tsuchida et al, 2018). In the soil drench method, seedlings were transplanted into pots with non-inoculated substrate and inoculated by pouring 30-mL of a blended inoculum slurry of *P. uncinulatum* isolates in sterile water into each pot (Tsuchida et al, 2018). In the agar plug method, transplanted seedlings were inoculated with three 5-mm agar plugs of *P. uncinulatum* isolates at the base of the seedling and secured with parafilm (Matsuura et al, 2010). The last method consisted of growing *P. uncinulatum* isolates in a mixture of sterile vermiculite, oats, and clarified V8 juice for 4-weeks and mixing into a soil-potting mix, and transplanting seedlings into the inoculated mixture (Davis et al, 1995). These methods were tested with three Romaine lettuce varieties (Duquesne, Patton, and 22PT/02) and inoculum consisted of a mix of three previously sequenced pathogenic *P. uncinulatum* isolates. All *P. uncinulatum* isolates were grown on CMA agar for 7-14 days old. Mock-inoculated plants served as controls, and the corresponding inoculum (slurry, agar plugs, vermiculite) were prepared with non-inoculated agar CMA plates. Each treatment was replicated six times. Overall, plants were inoculated at transplant 4-weeks post-emergence and maintained in growth chambers with a 12-hour day/12-hour night cycle at approximately 75% relative humidity and watered as needed. Plants were kept in a randomized block design. The number of infected plants and root biomass measurements were taken 1-week post-inoculation based on symptom development for all methods, excluding vermiculite (evaluated 8 weeks post-inoculation). A two-way T-test was performed comparing the root biomass of inoculated plants to mock-inoculated controls for each variety*inoculation method factorial treatment. Data were analyzed in RStudio.

<u>Objective 3- Characterize resistance to Verticillium wilt Races 1 and 2 and develop improved lettuce germplasm.</u>

Complete resistance to Verticillium wilt Race 1 is conferred by *Vr1*, but no germplasm has been identified that completely prevents infection by Race 2. New isolates of Race 2 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 2022-2023, our work focused on combining *Vr1* from RH12-3196 and resistance to Race 2 from

PI 171674 into a single genotype. Breeding lines were evaluated for resistance to Race 1 in two infested field experiments (planted April and May 2022) and screened with the *Vr1*-linked molecular marker. Germplasm was evaluated for resistance to Race 1, Race 2, and a mixture of Races 1 and 2 in growth room and greenhouse experiments. All germplasm was evaluated for horticultural traits and severity of vascular discoloration. We are finalizing selections for germplasm release in late 2023.

Objective 4- Develop germplasm with combined resistance to Fusarium wilt and Verticillium wilt.

Fusarium wilt emerged as a major problem in the Salinas Valley in 2019 and fields co-infected with both Fusarium and Verticillium Wilts have been identified since. In an effort to combine Fusarium and Verticillium wilt resistances into a single genotype, we are evaluating progeny segregating for Verticillium Race 1 resistance from RH12-3196 and Fusarium Race 1 resistance from 15361 (F5, King Louie x Autumn Gold). Thirteen breeding lines and check varieties were evaluated for Fusarium resistance by Dr. Stephanie Slinski (Univ. Arizona) in a field experiment (planted September 2022) under natural infection in Yuma, AZ. The same germplasm was evaluated for Verticillium Race 1 resistance in an experiment under infested Verticillium race 1 field conditions in Salinas, CA. We are conducting final field and greenhouse evaluations in preparation for germplasm release.

In early 2022, we confirmed the presence of a variant isolate of Fusarium wilt in fields in the Salinas Valley. Since then, our Fusarium research efforts have shifted to also include germplasm evaluation in the greenhouse and growth room under Fusarium race 1 and variant isolates. We continue to work with Dr. Alex Putman (UC, Riverside) to characterize Fusarium isolates collected throughout California and Arizona production areas. We will continue to breed for combined resistance to Fusarium and Verticillium wilts and will begin breeding for broad-spectrum Fusarium resistance.

RESULTS AND DISCUSSION:

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

In June 2022, seed of 120 lines (including USDA breeding material and commercial germplasm from one company) 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. Analysis of Means (ANOMA) was performed to compare individual lines to the overall experiment mean. 14 USDA breeding lines and 9 commercial entries were significantly more resistant (lower AUDPS) than the overall mean. In August 2022, seed of 329 lines (including USDA breeding material, recombinant inbred line (RIL) mapping population, and commercial germplasm from four companies) was planted in a replicated experiment at Spence Farm under natural INSV infection. ANOMA was performed on AUDPS data and 16 USDA breeding lines and 15 commercial entries were significantly more resistant (lower AUDPS) than the overall mean. 10 USDA breeding lines and 5 commercial entries were significantly more resistant (lower AUDPS) than the overall mean in both field experiments. Selections were made on resistant USDA lines and incorporated into the breeding program.

Over the past two years of field and greenhouse testing, we identified 13 lines that had consistent resistant, intermediate, or susceptible INSV reactions. We conducted greenhouse experiments using manual inoculation and viruliferous thrips transmission to validate field results. In two experiments, each plant was treated with both manual inoculation of the virus and viruliferous thrips transmission in the greenhouse. In one experiment, each plant was treated with manual inoculation only in the growth chamber. All plants were rated weekly for 5 weeks for INSV symptom severity on a scale from 0-5 and combined into AUDPS, results are presented in Table 1. Greenhouse tests with both virus and thrips inoculation closely matched field INSV severity results. The resistant checks 'Eruption', 'Cavalry', and 'Ruben's Red' performed as expected. The susceptible checks 'Conquistador' and 'White Paris' performed as expected, but 'Defender' had an intermediate reaction. Little variation was seen amongst intermediate checks. On average greenhouse AUDPS values were significantly higher than the field. This could be due to increased thrips and virus pressure in an enclosed space. While magnitude of AUDPS changed significantly in the greenhouse versus the field, rank of entries did not significantly change. This was not the case with lines tested in the growth chamber under manual virus inoculation only. 'Ruben's Red' was the only resistant check that had low INSV severity AUDPS under the virus alone; both 'Eruption' and 'Cavalry' had significantly higher AUDPS than expected. 'Defender' and 'White Paris' were both the most susceptible, as expected, but 'Conquistador' had the same intermediate AUDPS as 'Eruption'. These differences in results could indicate the importance of including thrips transmission in reliable germplasm evaluation for INSV resistance. They could also indicate a level of virus resistance present in 'Ruben's Red' but absent in 'Eruption' and 'Cavalry'. We will continue to characterize differences in mechanism of resistant amongst out resistant checks in an effort to best deploy resistance in improved varieties.

Table 1. INSV severity AUDPS (combined value of five weeks of evaluations) of 13 USDA lines in the field under natural infection, in the greenhouse under manual virus inoculation and viruliferous thrips transmission, and in the growth chamber under manual virus inoculation only.

Line	Field (virus + thrips)	Greenhouse (virus + thrips)	Growth chamber (virus only)
Eruption	1.3	10.4	7.0
Cavalry	2.1	10.1	10.4
Ruben's Red	2.7	10.4	4.0
Beacon	4.7	12.0	9.4
Salinas	5.1	12.5	5.9
Pacific	5.9	13.8	6.3
Red Hot	8.9	12.3	13.6
USDA280	9.4	13.6	5.5
USDA288	9.8	11.8	6.8
White Paris	11.0	19.3	15.8
Flashy Troutback	12.0	10.4	8.4
Conquistador	12.2	15.0	7.0
Defender	13.6	13.1	14.1

Dr. Daniel Hasegawa (USDA-ARS, Salinas) tested the same germplasm under thrips pressure (no virus) in choice tests and no-choice tests under controlled greenhouse conditions. In choice tests, thrips had the ability to choose the host plant that it feeds on. The number of adult thrips

present on each plant represents a potential difference in feeding preference. The number of immature thrips present on each plant represents a potential difference in reproduction preference. Feeding damage was assessed on the leaves with a score of 0-4. Analysis of Means (AMOVA) was performed on number of thrips. The number of adult thrips was significantly lower on 'Cavalry' and 'Eruption' suggesting they have a lower feeding preference (Figure 3). The number of adult thrips was significantly higher on 'Beacon' suggesting it has a higher feeding preference. The number of immature thrips was significantly lower on 'Cavalry', 'Eruption', and the USDA breeding lines USDA280 (RH15-0973) and USDA288 (RH15-0981) suggesting they have a lower reproduction preference. The number of immature thrips was significantly higher on 'Beacon', 'Conquistador', 'Pacific', 'Red Hot', and 'White Paris' suggesting they have a higher reproduction preference. For thrips feeding damage score, 'Pacific' was the only line to have a significantly different (higher) value than the overall mean. No lines were significantly lower than the mean.

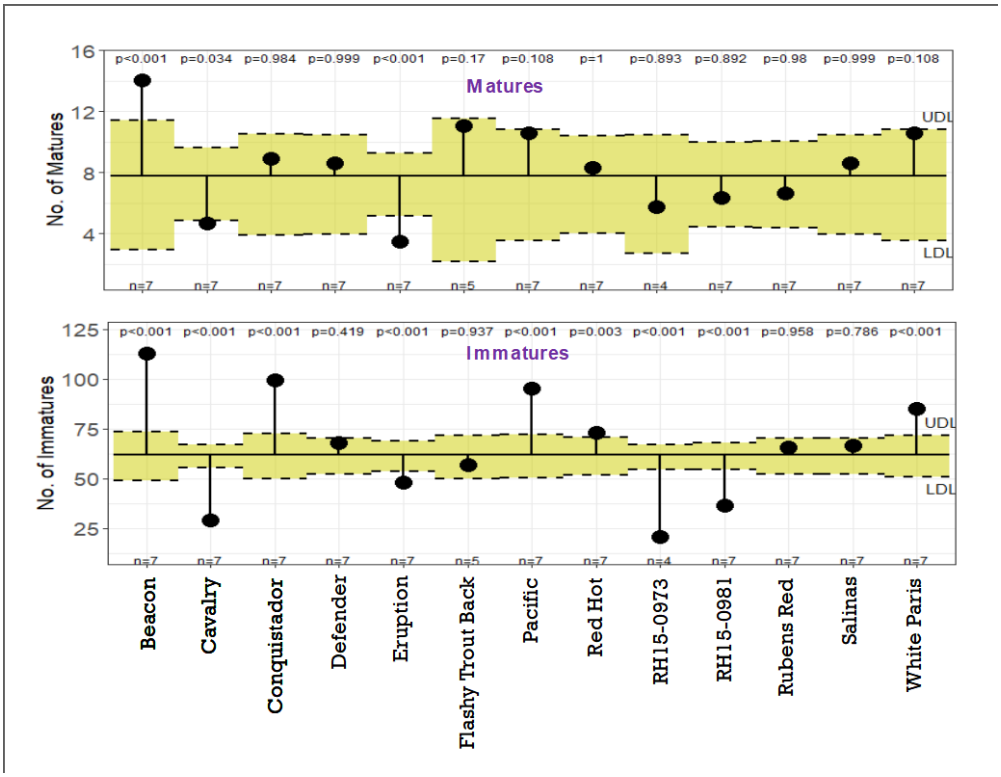

Figure 3. Analysis of Means (AMOVA) results of thrips only (no virus), choice tests of 13 USDA lines. Horizontal (center) line denotes overall mean. Area filled with color indicates 95% confidence interval of overall mean (UDL=Upper decision limit; LDL=Lower decision limit). If count data falls outside of the decision limits, then the test indicates that there is a statistical difference between count on that cultivar with the overall mean.

In no-choice tests, thrips were forced to feed on a leaf of each of the 13 lines. At the end of the experiment, each leaf was scored (0-4) for feeding damage and the number of feeding spots recorded. AMOVA was run on feeding score and number of spots. No cultivar statistically differed from the overall mean for feeding score in no-choice tests. For number of feeding spots, 'Beacon' was significantly higher than the overall mean and 'Flashy Troutback' and 'Ruben's

Red' were significantly lower than the mean (Figure 4). Further tests are underway to connect the significance of thrips-only tests to field results.

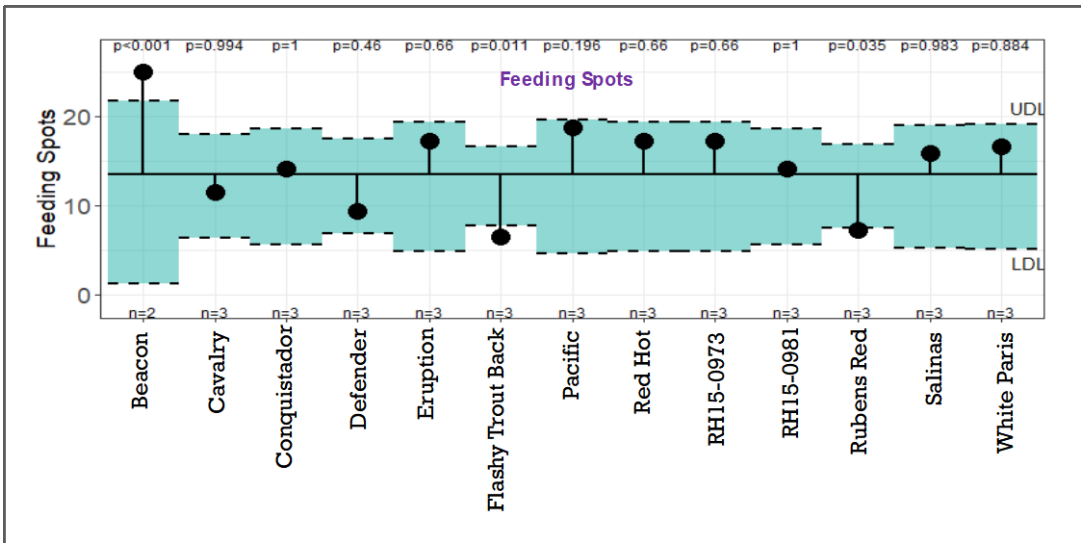

Figure 4. Analysis of Means (AMOVA) results of thrips only (no virus), no-choice tests of 13 USDA lines. Horizontal (center) line denotes overall mean. Area filled with color indicates 95% confidence interval of overall mean (UDL=Upper decision limit; LDL=Lower decision limit). If count data falls outside of the decision limits, then the test indicates that there is a statistical difference between count on that cultivar with the overall mean.

Objective 2- Develop a controlled-environment assay to evaluate germplasm resistance to Pythium.

Results from these experiments (Table 2) revealed that the rates of symptom development and disease progression vary by variety, and in general, are consistent with field performance observations. Of the varieties tested, the vast majority (n=11: BOS1566, BOS1687, Regency, Telluride, Momentous, ROM201, Red Fusion, Nun 00300, Primo, 22PT/01, San Andreas) showed high susceptibility to Pythium wilt with 98-100% of the plants showing root discoloration symptoms by 48-h post-inoculation. Other varieties (n=5: 22PT/08, Kodiak, Panoramis, Powerball, Paraiso) showed greater tolerance to Pythium wilt with an average of 90% of symptomatic plants by 48-h post-inoculation. On the other hand, Patton was the variety that showed the greatest tolerance by 48-h post-inoculation with only 55% of symptomatic plants. Additionally, varieties further varied in the number of dead plants characterized for being completely necrotic and wilted. For example, varieties such as Patton, 22PT/08, Kodiak, Momentous, Primo, and 22PT/01 had less than 55% of dead plants by 120-h post-inoculation, suggesting that while plants were symptomatic, the disease progressed more slowly. On the contrary, varieties BOS1566, BOS1687, Regency, ROM201, Red Fusion, and Nun 00300 had a 90% of dead plants dead by the 120-h post-inoculation evaluation. Other varieties such as Panoramis, Powerball, Paraiso, Telluride, and San Andreas were ranked as intermediate with an average of 68% of dead plants by the end of the evaluations. In general, this study demonstrated that, consistent with field experiments, plant infection and symptom development started appearing quite fast, and the number of Pythium-infected plants increased over time becoming more noticeable. By the last evaluation (120-h post-inoculation), symptoms were observed across nearly all plants, though some varieties were able to hold up more than others and not all

the symptomatic plants were dead by the end of the experiment. However, no true resistance (zero disease) was found among the seventeen varieties tested. Overall, this study validates the usage of the *in-vitro* assay as an additional tool for screening commercial lettuce varieties for tolerance to Pythium Wilt.

Table 2. Percent of symptomatic (root discoloration) and dead (necrotic and wilted) plants observed across lettuce commercial varieties inoculated with *Pythium uncinulatum* in an *in-vitro* assay.

Variety	Hours post-inoculation	Symptomatic (%)	Dead (%)
Patton	48	55.56	0.00
	72	90.28	15.28
	96	94.44	30.56
	120	100.00	48.61
22PT/08	48	88.89	0.00
	72	98.61	15.28
	96	98.61	40.28
	120	100.00	50.00
Kodiak	48	91.67	0.00
	72	97.22	8.33
	96	98.61	27.78
	120	100.00	33.33
Panoramis	48	94.44	0.00
	72	95.83	31.94
	96	100.00	66.67
	120	100.00	79.17
Powerball	48	91.67	8.33
	72	100.00	30.56
	96	100.00	58.33
	120	100.00	69.44
Paraiso	48	90.28	1.39
	72	97.22	13.89
	96	97.22	41.67
	120	98.61	61.11
BOS1566	48	98.61	0.00
	72	100.00	51.39
	96	100.00	77.78
	120	100.00	84.72
BOS1687	48	98.61	5.56
	72	98.61	47.22
	96	100.00	63.89
	120	100.00	84.72
Regency	48	100.00	9.72
	72	100.00	68.06
	96	100.00	84.72
	120	100.00	95.83
Telluride	48	98.61	0.00
	72	98.61	5.56
	96	100.00	38.89

	120	100.00	75.00
Momentous	48	98.61	4.17
	72	100.00	8.33
	96	100.00	25.00
	120	100.00	55.56
ROM201	48	100.00	5.56
	72	100.00	50.00
	96	100.00	79.17
	120	100.00	95.83
Red Fusion	48	100.00	11.11
	72	100.00	40.28
	96	100.00	83.33
	120	100.00	94.44
Nun 00300	48	100.00	4.17
	72	100.00	38.89
	96	100.00	80.56
	120	100.00	86.11
Primo	48	100.00	0.00
	72	100.00	6.94
	96	100.00	13.89
	120	100.00	20.83
22PT/01	48	100.00	8.33
	72	100.00	12.50
	96	100.00	38.89
	120	100.00	45.83
San Andreas	48	100.00	2.78
	72	100.00	13.89
	96	100.00	51.39
	120	100.00	58.33

Plants inoculated via dip, drench, and agar plug were harvested within 7-days due to early symptom development. Plants were harvested when the majority of inoculated plants within a method were symptomatic showing extensive root necrosis, and reduction of root biomass. Plants inoculated with vermiculite required a longer incubation period and were harvested after 8-weeks of no symptom development. At least 50% of plants of all variety*inoculation factorial treatment were evaluated under the microscope for the presence/absence of *P. uncinulatum* oospores. Symptomatic plants were prioritized if present, otherwise, asymptomatic plants were randomly selected. Roots of symptomatic-infected plants were often too decayed to be further evaluated. A total of 22% (n=25) of symptomatic plants were confirmed infected with Pythium wilt. Oospores were not observed in the root tissues of mock-inoculated plants. In general, all inoculation methods resulted in infected plants with the exception of vermiculite (Table 3). This was likely due to the lack of production of aerial mycelium and difficulty in colonizing the vermiculate mix. Results from these experiments also suggest that the most effective inoculation method is the agar plug (81% of inoculated plants developed symptoms, of which 20% were confirmed infected with Pythium wilt). This is likely due to the direct placement of the inoculum on the plant tissue. However, this method is also the least biologically relevant as field infection takes place through the roots rather than aboveground tissues. Drench treatment resulted in a higher frequency of infected plants, faster symptom development, and lower plant biomass

compared to the other inoculated treatments or mock-inoculated plants that remain asymptomatic. Marginal differences were observed between inoculated and mock-inoculated plants in the root dip treatment, suggesting this treatment should be further tested but increasing the inoculation exposure time.

Table 3. Counts of Inoculated and Infected Plants.

Method	Variety	Plant Count		Symptomatic Plants	Confirmed Infected Plants
		Control	Inoculated		
Agar Plug	Duquesne	18	18	15	2
	Patton	18	18	15	5
	22PT/02	18	18	14	2
Dip	Duquesne	12	12	9	1
	Patton	12	12	10	1
	22PT/02	11	12	10	0
Drench	Duquesne	14	14	14	4
	Patton	15	14	14	5
	22PT/02	13	15	14	5
Vermiculite	Duquesne	8	4	0	0
	Patton	13	10	0	0
	22PT/02	14	13	0	0
Total	-	166	160	115	25

In addition to inoculation, variation was observed among lettuce varieties likely due to differences in susceptibility and in symptom expression among them. Variety 22PT/02 was less responsive to any of the inoculation methods, and Patton showed marginal differences. Strong differences were observed for Duquesne variety, indicating that this variety is more susceptible to infection, consistent with field observations (Figure 5). For example, there was a statistically significant difference between the average root biomass of plants inoculated via agar plug of the Duquesne (p-value=3.3e-05) and Patton (p-value=0.028) varieties (Figure 1). There was no statistically significant difference of root biomass of inoculated plants compared to mock-inoculated controls of the 22PT/02 variety for any method. This research was presented at the California Plant and Soil Science Conference in Fresno CA in January 2023 and was ranked 3rd place in the graduate division.

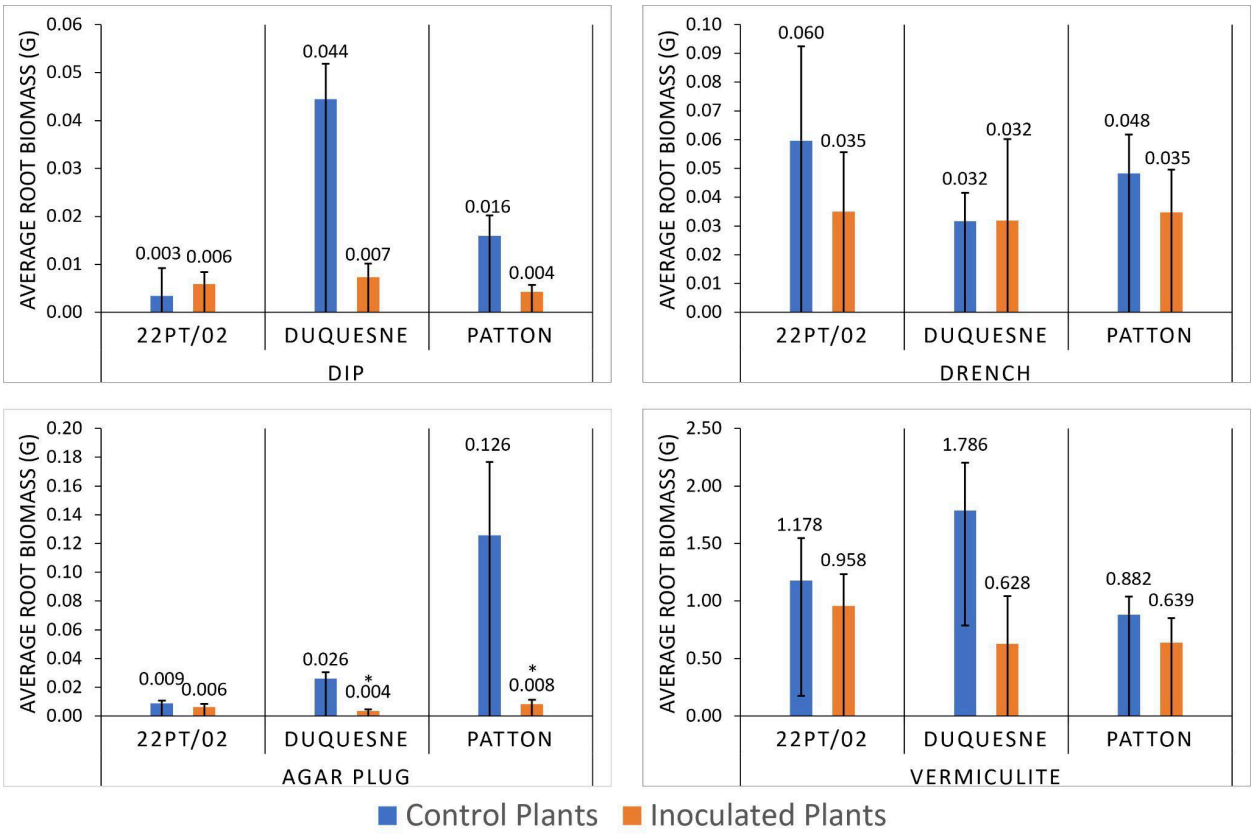

Figure 5. Comparison of Average Root Biomass by Inoculation Method. Statistical differences between inoculated and non-inoculated treatments are denoted with *.

Future (ongoing) studies will aim at optimizing the most effective inoculation method, improving the reproducibility and development of the most infected plants, and assessing plants at different developmental stages.

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

USDA breeding lines to combine Race 1 and Race 2 resistance and check entries were evaluated in the greenhouse under inoculation with Verticillium Race 1, Race 2, or an equal parts mixture of Races 1 and 2. All individuals were screened with the *Vr1*-linked molecular marker. 14 breeding lines that were *Vr1*-positive and had low vascular discoloration scores (0-5) under both Races were selected and advanced in the breeding program (Figure 6).

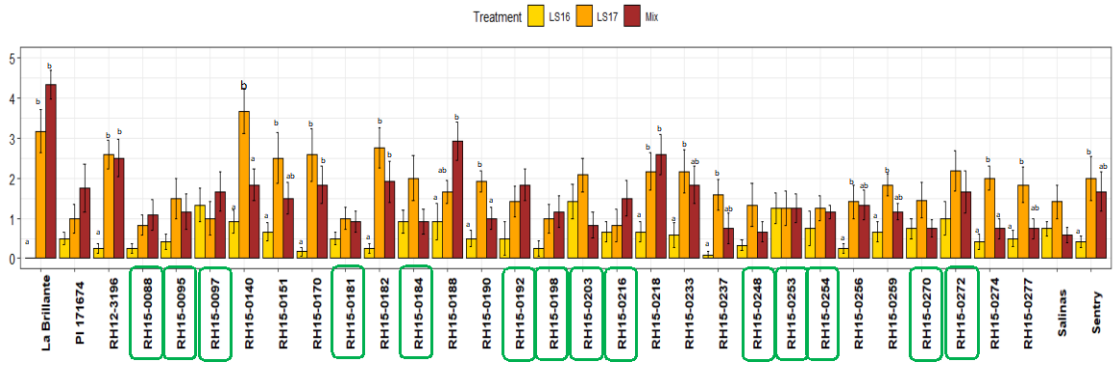

Figure 6. Vascular discoloration score (0-5) of USDA breeding lines and check entries inoculated in the greenhouse with Verticillium Race 1, Race 2, or an equal parts mixture of Races 1 and 2. Lines outlined in green were advanced in the breeding program.

The same germplasm was evaluated in the field under Verticillium Race 1 conditions in two experiments. Seven breeding lines with low vascular discoloration scores (0-5) under Race 1 field conditions were selected and advanced in the breeding program (Figure 7). We are finalizing selections for germplasm release in late 2023.

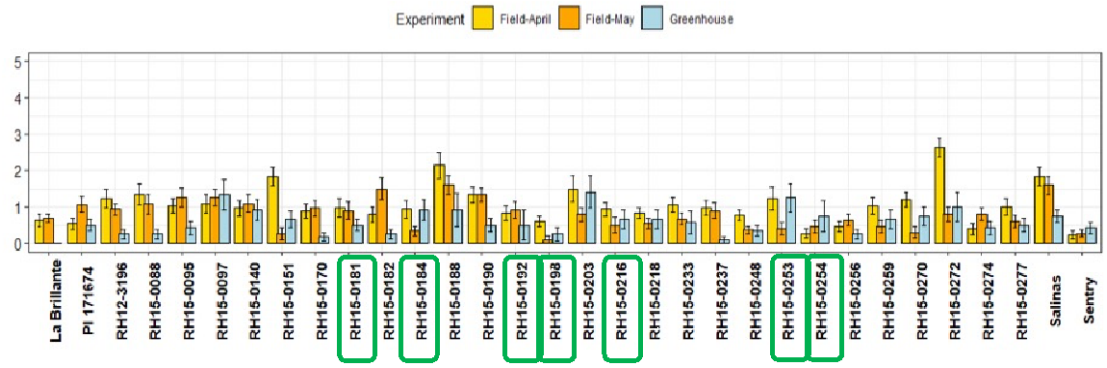

Figure 7. Vascular discoloration score (0-5) of USDA breeding lines and check entries under Verticillium Race 1 field conditions in two experiments compared with greenhouse Race 1 results. Lines outlined in green were advanced in the breeding program.

<u>Objective 4- Develop germplasm with combined resistance to Fusarium wilt and Verticillium wilt.</u>

Thirteen breeding lines to combine Fusarium and Verticillium wilt resistances and check varieties were evaluated for Fusarium resistance by Dr. Stephanie Slinski (Univ. Arizona) in a field experiment under natural infection in Yuma, AZ. Fusarium pressure was low in 2022, so reliable wilting and vascular discoloration scores were not taken. Horticultural notes on bolting and plant type were recorded. We will plant the trial again in 2023. The same germplasm was evaluated for Verticillium resistance in two experiments under infested Verticillium race 1 field conditions in Salinas, CA. 3 USDA breeding lines had the same or better vascular discoloration scores than the resistant check ‘La Brillante’. We are conducting final field and greenhouse evaluations in preparation for germplasm release.

Since we confirmed the presence of a variant isolate of Fusarium wilt in fields in the Salinas Valley in early 2022, our Fusarium research efforts have shifted to also include germplasm evaluation in the greenhouse and growth room. We developed and validated high-throughput

protocols to evaluate germplasm under Fusarium race 1 and variant isolates. We continue to evaluate commercial varieties and differential lines to characterize the impact of a new Fusarium race in the area.

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