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

BREEDING CRISPHEAD AND LEAFY LETTUCE

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SUMMARY

The program continues to emphasize the identification and incorporation of genes for disease resistance, particularly to downy mildew, and *Verticillium* and *Fusarium* wilts, into crisphead and the four leaf types suitable for California. Resistance for downy mildew is being introduced from 39 new sources. We have continued to monitor variation in the ability of the downy mildew pathogen to overcome resistance genes. New sources of resistance in our breeding program are more effective than the known resistance genes, but some super virulent isolates have been detected. Deployment of multiple new sources in combination will minimize the chances that changes in the pathogen will render all cultivars susceptible simultaneously. We have identified a major locus for resistance to *Fusarium*. We have implemented programs for resistance to INSV and *Globisporangium* (*Pythium) uncinulatum.* We have cloned all five genes of INSV and are screening germplasm for reaction to *Agrobacterium*-mediated transient expression of INSV proteins. Several lines exhibited recognition of one of more proteins. These lines have been shared with collaborators for testing with live virus. We have sequenced 70 isolates of INSV and assessed variation. We aim to sequence ~100 isolates per year. We have developed an assay to screen for resistance to *G. uncinulatum* and identified a few lines that exhibit markedly delayed symptoms.

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ABSTRACT:
The program continues to emphasize the identification and incorporation of genes for disease resistance, particularly to downy mildew, and *Verticillium* and *Fusarium* wilts, into crisphead and the four leaf types suitable for California. Resistance for downy mildew is being introduced from 39 new sources. We have continued to monitor variation in the ability of the downy mildew pathogen to overcome resistance genes. New sources of resistance in our breeding program are more effective than the known resistance genes, but some super virulent isolates have been detected. Deployment of multiple new sources in combination will minimize the chances that changes in the pathogen will render all cultivars susceptible simultaneously. We have identified a major locus for resistance to *Fusarium*. We have implemented programs for resistance to INSV and *Globisporangium* (*Pythium) uncinulatum.* We have cloned all five genes of INSV and are screening germplasm for reaction to *Agrobacterium*-mediated transient expression of INSV proteins. Several lines exhibited recognition of one of more proteins. These lines have been shared with collaborators for testing with live virus. We have sequenced 70 isolates of INSV and assessed variation. We aim to sequence ~100 isolates per year. We have developed an assay to screen for resistance to *G. uncinulatum* and identified a few lines that exhibit markedly delayed symptoms.

CONTINUING OBJECTIVES:

1) To identify new genes for disease resistance in wild germplasm and incorporate multiple genes from diverse sources into advanced crisphead and leafy breeding lines to maximize the likelihood of durable resistance.
2) To monitor variation in pathogen populations, particularly downy mildew and INSV, to facilitate the deployment of effective resistance genes.
3) To utilize the genetics of agriculturally important traits, particularly disease resistance.
4) To release advanced crisphead and leafy breeding lines that have resistance to multiple diseases, superior appearance and quality, high yielding ability, uniform maturity, and are slow bolting.

PROCEDURES AND RESULTS:

Development of Disease Resistant Lines

Breeding strategy

The overall strategy used in the UC Lettuce Breeding Program continues to be to initiate crosses and grow early generations at Davis; later generations are trialed and selected at several different lettuce-growing areas in collaboration with the USDA, Cooperative Extension in Salinas, Stephanie Slinski in Yuma, and California growers. Backcross and/or single-seed descent strategies are employed for most of the early generations. We select for type, color, slow bolting, and yield as well as disease resistance in the crisphead and the four leaf lettuce plant types. When possible, we use different sources of resistance for each plant type. This will diversify the selection pressure on the pathogen. The use of multiple sources of resistance will tend to increase the longevity of each resistance gene and decrease the chances that a single change in the pathogen will render multiple lettuce types susceptible.

Cultivars representing each type were selected to be the recurrent parents in backcross programs to introgress resistance genes based on their horticultural type and performance in California, their status in the public domain, and the presence of additional disease resistance genes. We are currently using Salinas for crisphead, Green Towers for romaine, Tropicana for green leaf, Red Fox and Red Tide for red leaf, and Bibb and Buttercrunch for butterhead programs. We welcome suggestions and further input on the field performance of these and other potential recurrent parents. The recurrent parents used in the final generations of backcrossing will be adjusted to reflect the industry standards as they advance.

Introgression of resistance to downy mildew

We are continuing to develop crisphead and leafy lettuce lines with resistance genes from diverse sources to provide protection against LDM in California. We are focused on generating advanced breeding lines with new resistance genes identified through germplasm screens with contemporary virulent isolates. Mapping and characterization of the new sources are in progress to provide a pipeline of new genes for resistance. Backcrossing programs to introgress the next generation of genes for resistance to LDM into crisphead and leafy types is a continual ongoing process.

Each backcross breeding line is tested in each generation against a contemporary, highly virulent isolate and the resistant plants used as the resistant parent for the next generation. The isolate used for selection is changed during the backcross program to reflect variation in *B. lactucae* in the field. Table 1 summarizes the status of introgressions of resistance in our breeding program.

Table 1. Status of introgressions for resistance to LDM in the UC Davis lettuce breeding program.

Accession	*Lactuca*	Type	Breeding Line Status	Category
PI491000	*L. saligna*	Green Leaf	BC_7S_1 testing for Hm	Advan. Donor
CGN5309	*L. serriola*	Green Leaf	BC_6	Advan. Donor
AZER-854	*L.serriola*	Green Leaf	BC_6	Advan. Donor
GEOR-292	*L. serriola*	Green Leaf	BC_6	Advan. Donor
AZER-853	*L.serriola*	Green Leaf	BC_6	Advan. Donor
CHEC-147	*L. saligna*	Romaine	BC_5	Advan. Donor
05G1421	*L.serriola*	Butterhead	BC_5	Advan. Donor
AZER-840	*L.serriola*	Romaine	BC_5	Advan. Donor
AZER-843	*L.serriola*	Crisphead	BC_5	Advan. Donor
05G1411	*L.serriola*	Green Leaf	BC_4	Intermediate
CHEC-082	*L. serriola*	Crisphead	BC_4	Intermediate
CHEC-136	*L. saligna*	Red Leaf	BC_4	Intermediate
AZER-811	*L.serriola*	Romaine	BC_4	Intermediate
CHEC-101	*L. saligna*	Romaine	BC_3	Intermediate
CHEC-108	*L. saligna*	Green Leaf	BC_3	Intermediate
AZER-871	*L.serriola*	Butterhead	BC_3	Intermediate
KYRGY-247	*L. serriola*	Butterhead	BC_2	Early donor
CHEC-158	*L. saligna*	Green Leaf	BC_2	Early donor
RUS-635	*L.serriola*	Red leaf	BC_2	Early donor
CHEC-132	*L. saligna*	Green Leaf	BC_2	Early donor
RUSS-653	*L.serriola*	Butterhead	BC_2	Early donor
CGN13330	*L. saligna*	Romaine	BC_1	Early donor
GEOR-282	*L. serriola*	Butterhead	BC_1	Early donor
GEOR-284	*L. serriola*	Red Leaf	BC_1	Early donor
GEOR-288	*L. serriola*	Iceberg	BC_1	Early donor
CGN5157	*L. saligna*	Green Leaf	BC_1	Early donor
ARM09-158	*L. serriola*	Crisphead	BC_1	Early donor
ARM09-172	*L. serriola*	Crisphead	BC_1	Early donor
CGN5301	*L. saligna*	Romaine	F_1	Early donor
AZER-856	*L.serriola*	Butterhead	F_1	Early donor
AZER-867	*L.serriola*	Crisphead	F_1	Early donor
ARM09-184	*L. serriola*	Romaine	Crosses initiated	Early donor
ARM09-168	*L. serriola*	Green Leaf	Crosses initiated	Early donor
ARM09-183	*L. serriola*	Red Leaf	Crosses initiated	Early donor
RUS-647	*L. georgica*	Romaine	Crosses initiated	Early donor
ISR-356	*L. serriola*	Crisphead	Crosses initiated	Early donor
ISR-335	*L. serriola*	Romaine	Crosses initiated	Early donor
ISR-363	*L. serriola*	Green Leaf	Crosses initiated	Early donor
ISR-382	*L. serriola*	Butterhead	Crosses initiated	Early donor

We are genetically characterizing the chromosomal positions of resistance genes in our program. Once these genomic regions are identified, molecular markers will be released for MAS to select lines with multiple resistance genes that confer resistance to all Californian isolates. Release of lines with pyramids of several resistance genes should increase the durability of the individual genes and provide longer lasting resistance.

Downy Mildew Surveys

To ensure that we are breeding for resistance against contemporary virulence phenotypes (VP) of *B. lactucae* present in the western US, we continue to sample and phenotype isolates of *B. lactucae* in collaboration with seed companies, growers, and extension personnel. A total of more than 2,575 isolates have been characterized since 1982.

We continue to work closely with IBEB-US, a breeding company-coordinated group that nominates official races of *B. lactucae*. It is a subgroup of the International Bremia Evaluation Board (IBEB-G), which strives to standardize race denomination procedures and the core differential set of resistant cultivars used to characterize isolates of *B. lactucae.* Individuals/companies interested in participating in IBEB-US should contact Erron Haggard (E.Haggard@enzazaden.com). Currently, races are based on reactions on the EU-D differential set, which was adopted in 2022. As of 2023, there are nine official California races. However, races Bl: 1US to Bl: 6US have not been observed for many years; therefore, they are not of agricultural relevance and are not available for distribution and screening. B1: 7US has not been observed since 2017 but has not been officially retired. We have supplied isolates of candidate new races for ring tests by the companies. We are also participating in an IBEB-G subcommittee on determining resistance for lettuce/isolate combinations which exhibit necrotic symptoms and profuse sporulation or no necrosis and sparse sporulation. This subcommittee is documenting these interactions and discussing ways to standardize resistant/susceptible scores for these combinations.

Our sampling remains largely opportunistic and relies on collaborators to send us samples when available. The fall and winter weather was conducive to LDM. We received many samples throughout the year. Collaborations with Stephanie Slinski at the Yuma Center of Excellence for Desert Agriculture resulted in good sampling from Arizona fields again this year. LDM occurrence peaked in the beginning of 2024 and we are still processing some of those samples. Our sampling has been complemented by isolates collected from lettuce lines specifically planted to catch naturally occurring *B. lactucae* in trap crop nurseries (see below). Last fall, we had a planting in Yuma, AZ and we currently, we have plantings in Salinas, CA,

Between April 2023 and March 2024, we collected a total of 57 isolates of *B. lactucae* from three trap crop nurseries (see below) and received an additional 226 isolates from collaborators in California and Arizona. With many samples, we virulence phenotyped two per field and froze the rest. If the two samples showed very different virulence pathotypes, we phenotyped additional samples. So far, we have completed virulence phenotyping for 157 isolates, 32 from trap crops (25 CA and 7 AZ) and 121 from field collections (96 CA and 25 AZ). The vast majority of these isolates have novel VPs (Fig. 1).

While the frequency of novel virulence phenotypes (VPs) is high, we are still detecting low levels of Bl: 8US and Bl: 9US. The presence of Bl: 8US has increased in recent years. It is characterized by avirulence on *Rsal-1*, *R52, R54, R55, Dm11 + R57* (Kibrille), *R65,* and *Dm11 + R58* (RYZ20007). While the type isolate is avirulent for *Dm24 + Dm38* (RYZ910457), historically isolates virulent on RYZ910457 were included in Bl: 8US and this “progenitor” version is

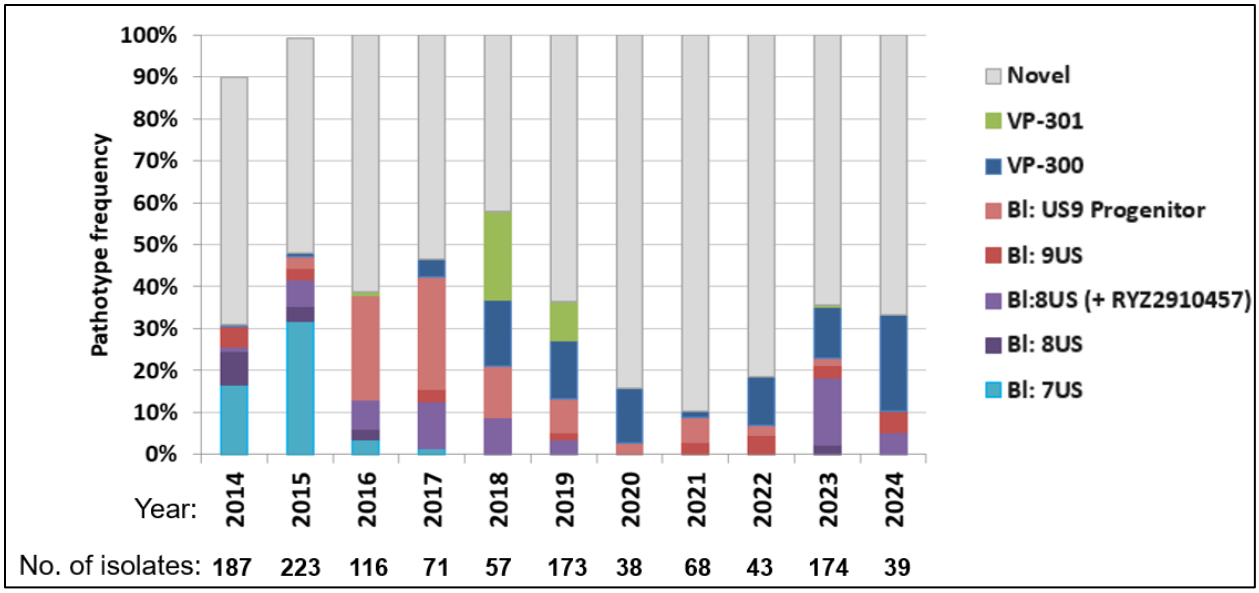
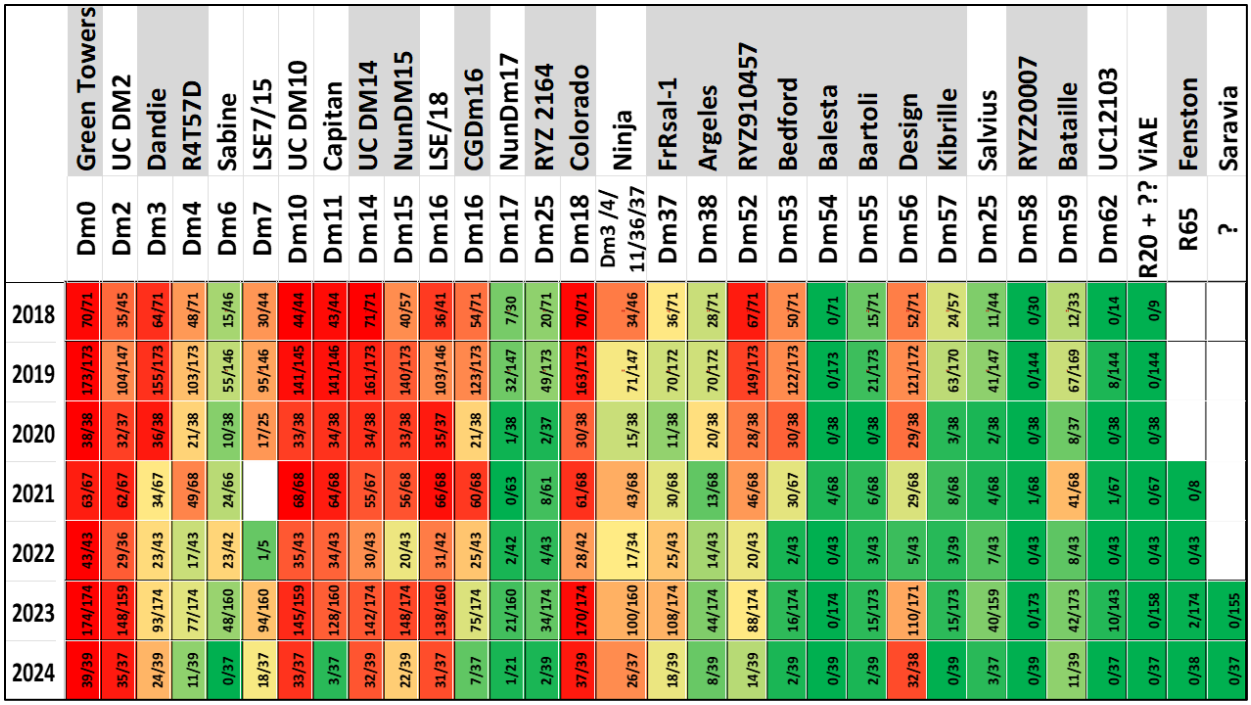


Figure 1. Frequency (%) of races detected in CA and AZ 2011–2023. Note that the 2024 data is based on only 39 isolates so far. Years 2021 and 2022 isolates were mostly from the trap crops.

currently the one present in the field. Bl: 9US has maintained a low presence and has avirulence on *Dm4, R38, Dm24 + Dm38* (RYZ2164), *R54, R55, Dm11 + R57* (Kibrille), *R65,* and *Dm11 + R58* (RYZ20007), as well as an incomplete avirulence on *R53*. Bl: US9 Progenitor has a similar phenotype to Bl: 9US but has not been filtered by culturing on Fr-RSal to stabilize the virulence phenotype. Virulence phenotype VP-300, which has been observed since 2017, was detected in multiple locations and multiple years and overcomes important *Dm* genes. It is characterized as being avirulent on *Dm6*, *Dm17*, *Dm25* (*n1*), *Dm54* (*n4*), *Dm55* (*r1*), and Kibrille (*Dm57 + Dm11*) and virulent on *Dm38* (Argeles), i.e., it is a "*Dm38* breaker". Candidate type isolates were distributed and ring tested in 2021 and 2022. At that time the companies voted not to nominate those candidate isolates as a race due to inconsistent scoring between companies and reduced frequency of VP-300. However, in 2023 VP-300 was the second most frequent VP, so the companies requested another type isolate be identified. In response, we will be evaluating and distributing candidate type isolates for ring testing this summer.

Of the novel pathotypes, there was no common VP other than VP300 progenitor. We continue to see some isolates with VPs that had rarely been seen before, such as virulence on FrSal-1 and RYZ2164 and/or Kibrille. However, there is no one new VP that is becoming prevalent.

Considering the data from 2022 through 2024, the newer lines, Balesta (*Dm54*), Bartoli (*Dm55*), RYZ20007 (*Dm58+Dm11*), and Fenston (*R65*), continue to be resistant with at most 4% of all isolates typed being virulent on these lines (Fig. 2). Saravia that is being tested for possible addition to the EU-D set, is also resistant to all isolates tested. In contrast, cv. Bataille (*Dm59*) was only resistant to about a quarter of the isolates (24%). Some of the isolates are losing virulence on some differential lines, notably Dandie (55%), R4T57D (41%), LSE7/15 (56%), and CGDm16 (42%), and RYZ910457 (48%). Virulence on Capitan had a sudden change from 80% to 8% in 2024, we will examine this further to determine if this a sampling or other artifact.



	Green Towers	UC DM2	Dandie	R4T57D	Sabine	LSE7/15	UC DM10	Capitan	UC DM14	NunDM15	LSE/18	CGDm16	NunDm17	RYZ 2164	Colorado	Ninja	FrRsal-1	Argeles	RYZ910457	Bedford	Balesta	Bartoli	Design	Kibrille	Salvius	RYZ20007	Bataille	UC12103	ViAE	Fenston	Saravia
	Dm0	Dm2	Dm3	Dm4	Dm6	Dm7	Dm10	Dm11	Dm14	Dm15	Dm16	Dm16	Dm17	Dm25	Dm18	Dm3 /4/ 11/36/37	Dm37	Dm38	Dm52	Dm53	Dm54	Dm55	Dm56	Dm57	Dm25	Dm58	Dm59	Dm62	R20 + ??	R65	?
2018	70/71	35/45	64/71	48/71	15/46	30/44	44/44	43/44	71/71	40/57	36/41	54/71	7/30	20/71	70/71	34/46	36/71	28/71	67/71	50/71	0/71	15/71	52/71	24/57	11/44	0/30	12/33	0/14	0/9		
2019	173/173	104/147	155/173	103/173	55/146	95/146	141/145	141/146	161/173	140/173	103/146	123/173	32/147	49/173	163/173	71/147	70/172	70/172	149/173	122/173	0/173	21/173	121/172	63/170	41/147	0/144	67/169	8/144	0/144		
2020	38/38	32/37	36/38	21/38	10/38	17/25	33/38	34/38	34/38	33/38	35/37	21/38	1/38	2/37	30/38	15/38	11/38	20/38	28/38	30/38	0/38	0/38	29/38	3/38	2/38	0/38	8/37	0/38	0/38		
2021	63/67	62/67	34/67	49/68	24/66		68/68	64/68	55/67	56/68	66/68	60/68	0/63	8/61	61/68	43/68	30/68	13/68	46/68	30/67	4/68	6/68	29/68	8/68	4/68	1/68	41/68	1/67	0/67	0/8	
2022	43/43	29/36	23/43	17/43	23/42	1/5	35/43	34/43	30/43	20/43	31/42	25/43	2/42	4/43	28/42	17/34	25/43	14/43	20/43	2/43	0/43	3/43	5/43	3/39	7/43	0/43	8/43	0/43	0/43	0/43	
2023	174/174	148/159	93/174	77/174	48/160	94/160	145/159	128/160	142/174	148/174	138/160	75/174	21/160	34/174	170/174	100/160	108/174	44/174	88/174	16/174	0/174	15/173	110/171	15/173	40/159	0/173	42/173	10/143	0/158	2/174	0/155
2024	39/39	35/37	24/39	11/39	0/37	18/37	33/37	3/37	32/39	22/39	31/37	7/37	1/21	2/39	37/39	26/37	18/39	8/39	14/39	2/39	0/39	2/39	32/38	0/39	3/37	0/39	11/39	0/37	0/37	0/38	0/37

Figure 2. Frequency of reactions to resistance genes observed from 2018 to 2024. The numbers indicate the number of isolates showing a virulent reaction out of the total number tested. Lines with grey highlighting are in the EU-D set. Red = predominantly virulent/susceptible reactions. Green = predominantly avirulent/resistant reactions.

In the last year, 154 isolates of *B. lactucae* have been characterized for mating type using the two molecular markers described in the 2021 CLGRB report. The majority (139) were B_2 mating type while only 15 were B_1. All B_1s were found from three fields in California, one of which also contained B_2 isolates. 143 isolates were typed for fungicide insensitivity. There were no isolates insensitive to Orondis or Revus. We continue to see a range of insensitivity to Metalaxyl (Fig. 3).

Trap Crop Nurseries

We continue to plant trap crop nurseries to assess the diversity of *B. lactucae* and to screen for isolates capable of breaking resistance genes in breeding lines. These nurseries are comprised of plots with approximately 57 resistant lettuce lines interspersed with rows of susceptible wild and cultivated lines, which serve as spreader rows. The resistant lettuce lines include donors for UC resistance breeding, cultivars in the EU-D differential series, UCD breeding lines, and lines of interest provided by collaborators. These plantings also serve as collection sites for isolates of *B. lactucae* from California and Arizona. Please contact us if you are willing to host a trap crop nursery or have lines you would like us to include.

This year, we planted one trap crop nursery in the Yuma in November 2023 and two in Salinas in June and July 2024. These trials were conducted in collaboration with Stephanie Slinski from the Yuma Center of Excellence for Desert Agriculture, Miguel Macias from Sakata Seed, and Ryan McMillen from BASF. Approximately 25 isolates were collected from our Yuma trial, representing a mixture of 8US, 9US, and novel races. Rating of the trap crops in Salinas is pending.

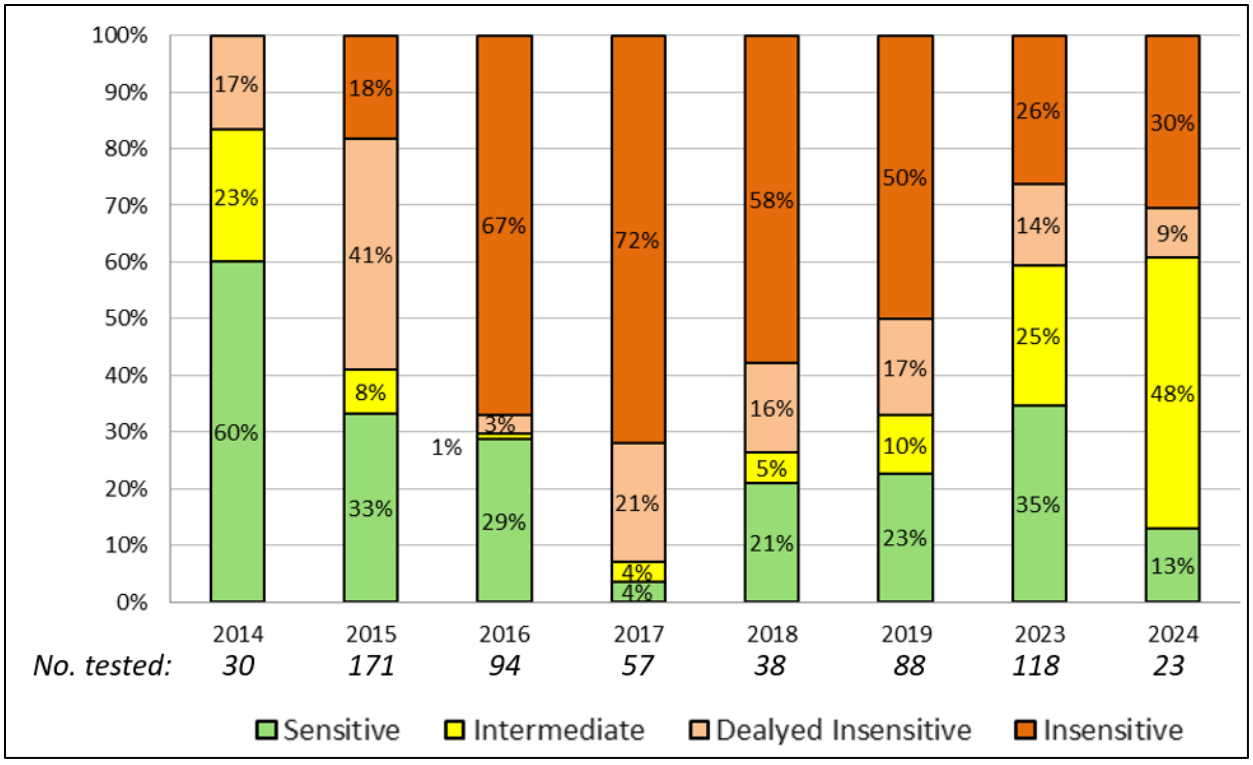


Figure 3. Insensitivity to metalaxyl in isolates from 2014 to 2024. Every year the isolates typed show a range of sensitivity to Metalaxyl. We do not have data for years 2020-2022.

Figure 4. Trap crop nursery in Yuma, AZ grown November 2023 - February 19, 2024.

Resistance to *Fusarium* wilt

We continued to collaborate with Dr. Stephanie Slinski at the Yuma Center for Excellence for Desert Agriculture, University of Arizona to trial lines in commercial fields for resistance to *Fusarium* wilt. In a diversity panel study of *Fusarium* resistance in Davis, CA in summer 2019 and in Yuma, AZ in winter 2020, we identified cultivars Reine de Glaces (RDG) and Delsay with contrasting levels of resistance/susceptibility to *Fusarium* (CLGRB breeding report 2020). Since 2021, we have trialed in Yuma a segregating population resulting from the cross of these two cultivars. Only 2021 and 2023 had good disease development, although seed germination problems in 2021 resulted in a skewness of the disease distribution towards apparent resistance (Fig. 5).

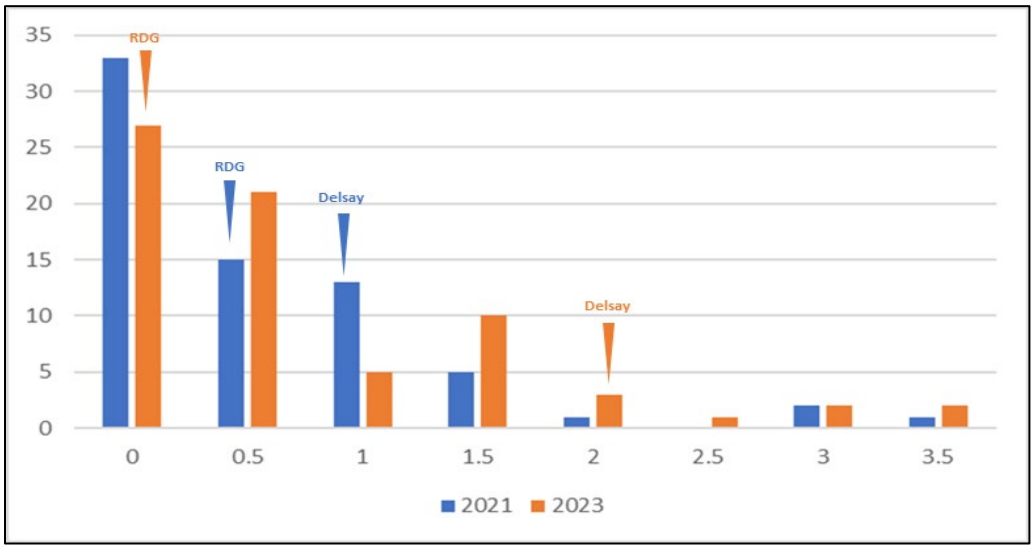


Figure 5. Segregation of resistance in the RDG x Delsay population in Yuma in 2021 and 2023. Disease scores are on the x axis (0 = resistant to 3.5 = susceptible). The y axis shows % infection.

We assessed the segregation of resistance to Fusarium in both 2021 and 2023 and conducted QTL analysis. We found a major significant QTL in 2023 on chromosome eight (Fig. 6, right panel) explaining 35.5 % of the phenotypic variance of resistance. A peak was observed in 2021 at the same position but it was not significant (Fig. 6, left panel).

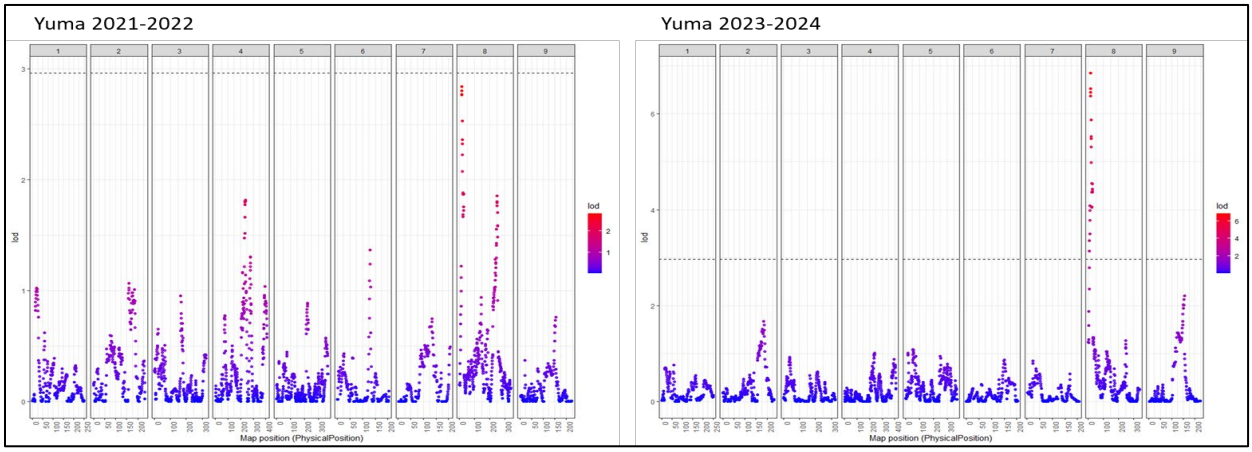


Figure 6. QTL analysis of resistance to Fusarium in the RDG x Delsay RIL population grown in Yuma in 2021 and 2023. The horizontal dotted line indicates the LOD significance threshold. Note the different scales of the LOD score on the y-axis.

Resistance to Impatiens Necrotic Spot Virus (INSV)

At the request of the CLGRB, we initiated a program to breed for resistance to INSV. As part of a one-year FFAR ROAR program award with matching support from the CLGRB, we cloned all of the open reading frames (ORFs) of INSV into a vector for *Agrobacterium*-mediated transient expression in lettuce leaves. We are screening lettuce germplasm for necrotic reactions in lettuce to the viral proteins encoded in INSV transiently expressed using *A. tumefaciens.* So far, we have screened 141 cultivated and wild *Lactuca* accessions. The vast majority showed no reaction, but few lines showed moderate or strong HR reaction to the viral proteins (Fig. 7). Some lines reacted to *Agrobacterium* alone and so their reaction to INSV could not be assessed using this protocol. We are continuing to screen more germplasm. We have shared 30 lines showing moderate or strong reactions for our collaborators, Kelly Richardson and Daniel Hasegawa (USDA Salinas), to test against live virus in greenhouse screens.

Figure 7: Reactions to transient expression of INSV genes in lettuce germplasm. Whole ~four-week-old plants were vacuum infiltrated with a pool of five strains of *Agrobacterium*, each expressing a different INSV gene. Example reactions of individual plants are shown; leaves are ordered from youngest to oldest. Note that the youngest leaves were not as developed at the time of infiltration. 107 lines showed no reaction; 21 showed a moderate reaction, and 13 showed a strong reaction.

It is important to determine and monitor the variability of INSV, particularly in the major lettuce production areas. The closely-related tomato spotted wilt virus (TSWV) is notoriously variable and resistance-breaking strains have evolved that overcome resistant lines of tomato or pepper. Existing sequence resources in GenBank for INSV are extremely limited. We found that the quickest, simplest, and most cost-effective strategy is to sequence cDNA libraries made from RNA extracted from infected lettuce leaves rather than primer-mediated amplicon sequencing. We can pool many samples per lane on an Element Biosciences Aviti sequencer. We plan on sequencing ~100 samples of INSV per year. We have so far received ~175 samples and have sequenced and assembled 70 samples; these originate from throughout California (many courtesy of Dr. S Koike, Trical Diagnostics). The proportion of viral reads varies widely from <1 to >25%; however, even with low viral loads, we have been able to assemble whole viral tripartite

genomes. Samples sequenced and analyzed to date have revealed variation in the Californian population of INSV; these samples were distinct from isolates from elsewhere and other plant hosts (Fig. 8). Sequence identity was more than 98.6% among CA/AZ isolates and more than 96.8% with five published isolates. Californian INSV isolates had only 64% identity with TSVW sequences.

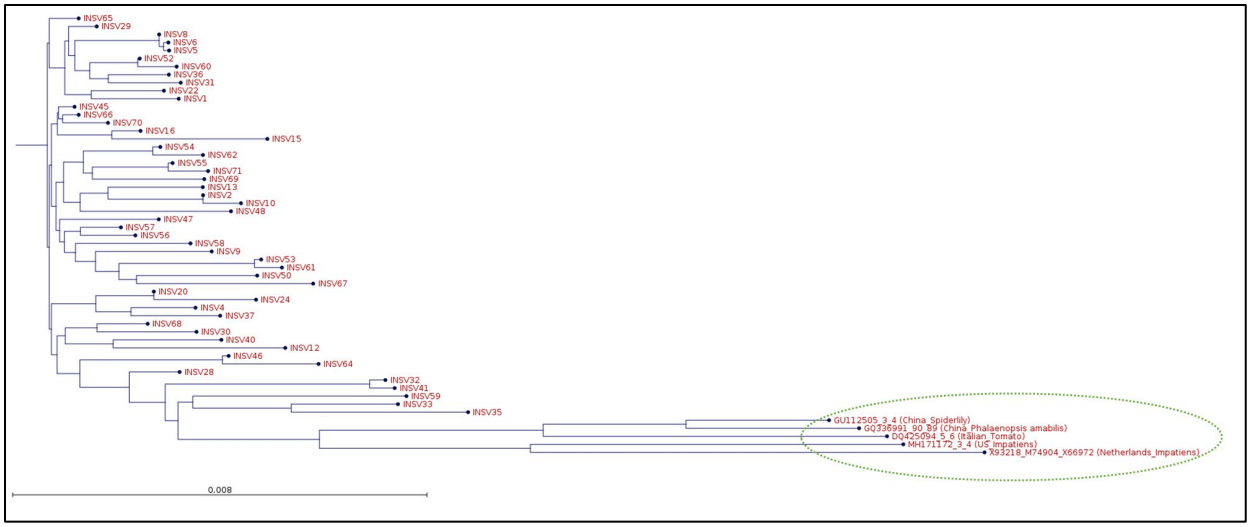


Figure 8. Neighbor joining phylogenetic tree of the genomes of 50 isolates of INSV from lettuce grown in California and five published sequences of INSV isolates from different hosts and regions (circled in green).

Resistance to *Globisporangium* (*Pythium) uncinulatum*

Pythium wilt of lettuce is caused by the now-named *Globisporangium uncinulatum*. This has recently become an increasing problem in the Salinas valley, frequently associated with INSV; therefore, on the request by the CLGRB, we initiated a program to breed for resistance to Pythium wilt in collaboration with Jose Pablo Dundore-Arias (JP), CSU Monterey Bay.

We are analyzing sequences of isolates from symptomatic roots in order to understand the diversity of *Globisporangium*(*Pythium*) spp. We have so far generated short reads for 19 isolates from the Dundore-Airas lab using the Aviti DNA sequencer. Comparative genomic analysis using k-mers for 14 of these isolates and existing sequences of other species of *Globisporangium* and *Pythium* grouped these isolates into four major clusters, with eight clustering with previously described sequences of *G. uncinulatum* (Fig. 9); the six other isolates clustered with different species. We are currently assembling the genomes of representatives of each cluster using PacBio HiFi and Oxford Nanopore long reads. We will annotate the genome of *G. uncinulatum* as a basis for generating diagnostics and monitoring its variation as well as an aid for evaluating resistance with validated isolates.

We developed an assay to assess the pathogenicity and host range of isolates from symptomatic roots as well as to screen for resistance to *G. uncinulatum*. *G. uncinulatum* and other isolates are grown on agar media for a week; then, 1-week-old lettuce seedlings are transferred from filter paper onto the cultures. Susceptible lines begin to show root discoloration starting on the second day post-transfer. The disease symptoms become increasingly severe over time and susceptible plants are completely dead ~seven days post transfer (Fig. 10). The pathogenicity of isolates from other groups was little or none in this assay. Several other plant species including

Arabidopsis, tomato, pepper, artichoke, sunflower, and daisy were tested to investigate the host range of *G. uncinulatum*. Arabidopsis, tomato, and pepper showed no disease symptoms when challenged with *G. uncinulatum*. Daisy and sunflower exhibited similar disease symptoms as lettuce, but disease progression was much slower. Artichoke showed the same level of susceptibility as susceptible lettuce cultivars. Therefore, the host range of *G. uncinulatum* seems to be restricted to the Compositae family.

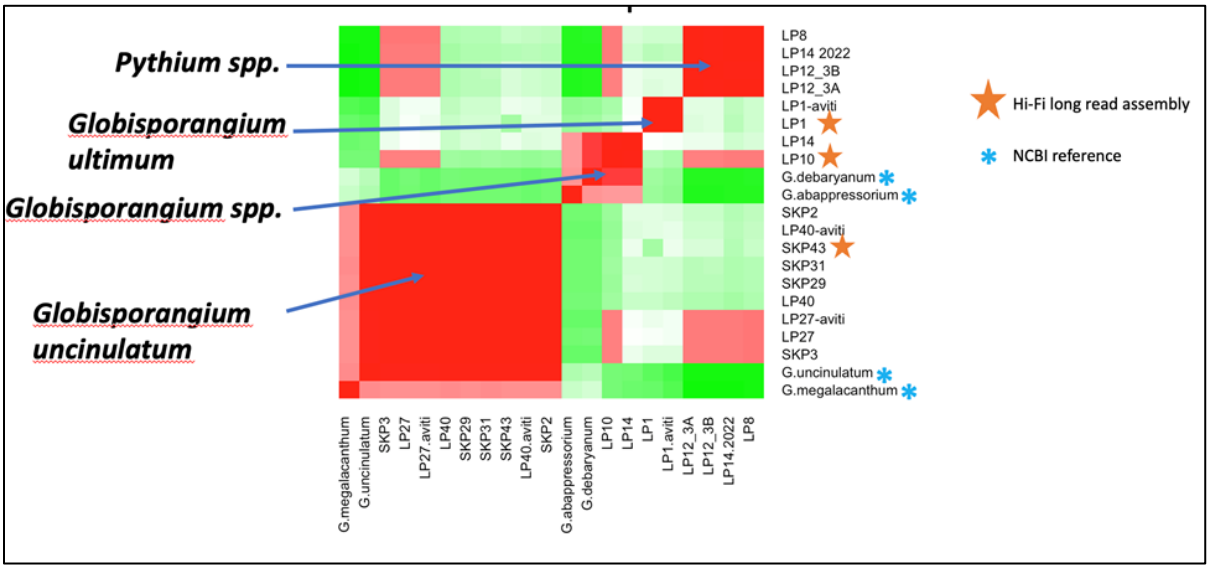


Figure 9. K-mer analysis of 14 isolates collected from roots of lettuce plants symptomatic for Pythium wilt disease.

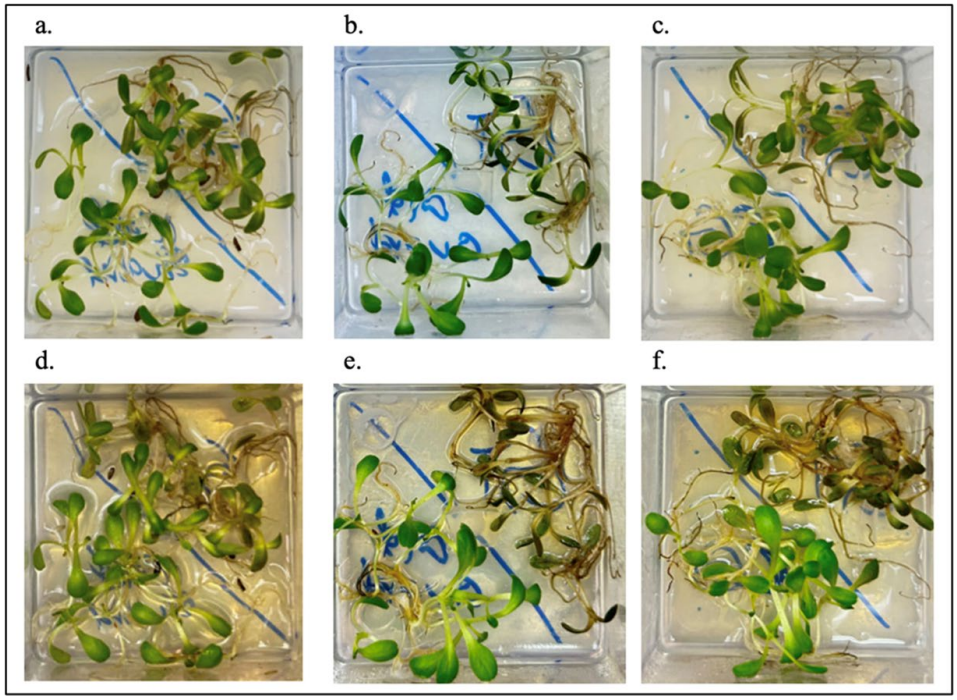


Figure 10. Cv. Coolguard and susceptible check, Green Towers, challenged with *G. uncinulatum* isolate SKP43. Three replicates with Coolguard bottom left and Green Towers right, (a)-(c): 3 d.p.i (d)-(f): 6 d.p.i. Symptoms are scored relative to Green Towers.

We have initiated screening germplasm with a validated isolate, SKP43, of *G. uncinulatum* using the same assay with Green Towers as the susceptible check. We are initially screening the parental lines of our mapping populations and then our germplasm collection for resistance to *G. uncinulatum*. We have so far screened 29 lines; 21 were as susceptible as Green Towers and 5 were less susceptible than Green Towers. One *L. serriola*, one *L. saligna*, and cv. Coolguard, exhibited greatly delayed symptoms and death (Fig. 10).

Supply of isolates

We have continued to supply contemporary California isolates of *B. lactucae* to breeding companies and other research groups. In particular, we have supplied type isolates of the nominated races as well as candidate isolates for ring tests in support of IBEB. We have trained personnel from the seed industry and others on how to work with *B. lactucae*.