

CALIFORNIA LEAFY GREENS RESEARCH PROGRAM
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REPORT

Project Title: Lettuce Breeding and Genetics (USDA)

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Abstract:

The lettuce industry of California requires continued development of improved, adapted cultivars to meet new disease and insect problems, changes in the market, and changes in growing procedures. The lettuce breeding and genetics project aims to incorporate valuable traits into crisphead, mixed lettuce, and spring mix cultivars and breeding lines that are adapted to coastal California and low desert production conditions. In parallel, we develop information and tools to increase the utility of our germplasm. In 2018 and 2019, we emphasized developing resistance to Verticillium wilt, lettuce drop, Fusarium wilt, corky root, downy mildew, orthotospoviruses, bacterial leaf spot, leafminer, lettuce aphid, as well as bolting resistance, extending fresh-cut salad shelf-life, and development of molecular markers for marker-assisted selection. In all programs, horticultural traits, adaptation, and resistance to tipburn were considered essential.

Objectives:

Develop landmark cultivars, advanced breeding lines, information, and tools for use by other breeders, scientists, producers and growers. Address problems facing the lettuce industry using genetic approaches that are suited to coastal, desert, and interior valley locations, to the various types of lettuce, and to the demands of different production and marketing approaches.

Objective	Page
Lettuce Drop	2
Fusarium Wilt	4
Impatiens necrotic spot virus	10
Bacterial Leaf Spot	11
Leafminer	12
Lettuce Aphid	14
Tipburn	15
Postharvest deterioration of fresh-cut lettuce	17
Molecular Marker Development	19
Recent Publications.....	20

Procedures and Results:

LETTUCE DROP (in collaboration with *B. Mamo, R. Hayes, M. Truco, K. Puri, R. Michelmore,* and *K. Subbarao*)

Lettuce drop, caused by *Sclerotinia minor* and *S. sclerotiorum*, is an economically important disease of lettuce. The association of resistance to lettuce drop with the commercially undesirable trait of fast-bolting has previously hampered the integration of host resistance in control of this disease. Eruption is a slow-bolting cultivar (cv.) that exhibits a high level of resistance to lettuce drop. Eruption also is completely resistant to Verticillium wilt caused by race 1 of *Verticillium dahliae*. A recombinant inbred line population from the cross Batavia Reine des Glaces × Eruption was genotyped by sequencing and evaluated for lettuce drop and bolting in a field infested with *S. minor* in three experiments and for incidence of Verticillium wilt. Two quantitative trait loci (QTL) for lettuce drop resistance were consistently detected at least in two experiments and two more QTL in another experiment; the alleles for resistance at all four QTL originated from Eruption (Figure. 1). Three out of four resistance QTL are distinct from QTL for bolting. QTL for lettuce drop resistance in Eruption also is distinct from QTL for red pigmentation due to anthocyanin accumulation. Resistance to Verticillium wilt in Eruption is conferred by the *Verticillium resistance 1 (Vr1)* locus previously described from cv. La Brillante. The SNP markers linked with these QTL will be useful in breeding for resistance through marker-assisted selection.

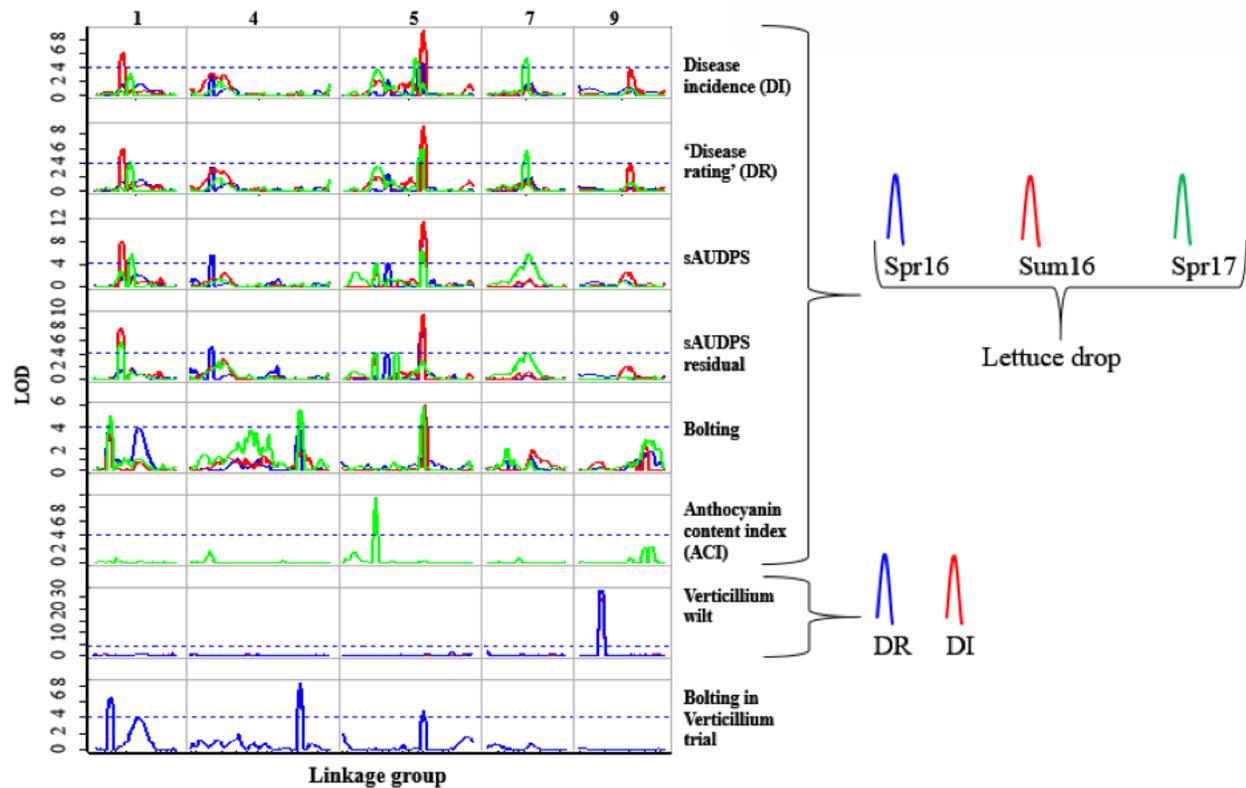


Figure 1. Distribution of QTLs for resistance to lettuce drop, Verticillium wilt race 1, rate of bolting, and anthocyanin content index identified in the Reine des Glaces × Eruption recombinant inbred line population. Detailed information is provided in the paper accepted for publication in Theoretical and Applied Genetics.

Breeding lines with partial resistance to lettuce drop:

Eight romaine breeding lines with a partial resistance to lettuce [drop](#) were developed from a cross between romaine cv. Hearts Delight (susceptible to the disease) and Latin type cv. Eruption (partially resistant to the disease – locations of QTLs for resistance on molecular linkage map is in Figure 1). Results of field tests indicate that the breeding lines have a partial resistance mostly in the range between the two parental lines. Additional field trials are in progress to evaluate their horticultural traits and resistance to other biotic and abiotic factors.

Table 1. Disease resistance (proportion of diseased plants) and horticultural characteristics of eight breeding lines and their parents.

Name	Lettuce drop [incidence]	Head weight [kg]	Head height [cm]	Core height [cm]	Tipburn [incidence]	Salad shelf-life [0-10] ^z
Eruption	0.25	0.51	16.7	6.9	0.57	6.4
Hearts Delight	0.74	1.38	31.9	9.6	1.00	5.2
RH12-3038	0.48	1.39	29.6	8.8	0.97	5.3
RH12-3039	0.47	1.50	32.0	10.0	1.00	5.3
RH12-3057	0.64	1.14	29.7	9.5	0.93	5.4
RH12-3058	0.62	1.06	31.0	9.1	1.00	6.1
RH12-3059	0.59	1.15	30.2	8.7	0.90	4.7
RH12-3060	0.79	0.93	30.2	9.9	0.90	5.2
RH12-3061	0.59	1.31	30.9	9.9	0.90	6.0
RH12-3080	0.58	1.13	29.6	8.2	0.90	3.7

^zShelf-life of fresh cut lettuce was scored on the scale from 0 to 10, where lower values indicate better shelf-life (less deteriorated tissue after storing at 4°C for six weeks)

FUSARIUM WILT (in collaboration with M. Matheron, S. Koike, and T. Turini)

Fusarium wilt (FW) incited by *Fusarium oxysporum* f.sp. *lactucae* race 1 was first reported in the Huron, CA Fall season lettuce production area in the 1991 (Hubbard and Gerik, 1993). Growers there were able to shift to different fields, thereby out-manuevering the pathogen once it was identified in a particular field. Lettuce production in Huron decreased during the recent drought years. FW was first observed in Arizona in 2001 (Matheron et al., 2005) when six commercial fields were infected in the Yuma area, where it continues to be a major production limiting factor. FW occurred in the Pajaro Valley as early 2002, but was controlled through rotation with strawberry plantings that were always fumigated with a combination of methyl bromide and chloropicrin for soilborne disease, insect, and weed control. FW is reportedly present in various parts of Salinas Valley, where it is apparently becoming more widespread. Four races have been reported to date; races 1, 2 and 3 occur in Japan (Fujinaga et al., 2003); races 1 and 4 occur in Europe (Gilardi et al., 2017). Data to date indicate the present of FW race 1 in Arizona and California. Anecdotal reports indicate co-infection of lettuce by Fusarium wilt and Verticillium wilt in various fields in Salinas Valley.

This project used the FW-resistant romaine lettuce ‘King Louie’ (Matheron et al., 2005) as a source of resistance to develop FW-resistant iceberg lettuce for early-Fall season lettuce production in the Yuma production area (see germplasm release below). We evaluated in 2018 six advanced (F5) FW-resistant selections in two naturally-infected field trials in Salinas Valley. The FW race differentials were included in order to characterize the pathogen.

The highly susceptible reaction of ‘Patriot’ and resistant reaction of ‘Costa Rica No. 4’ indicated presence of race 1 (Table 2) in Salinas Valley and that Verticillium was likely not present in the field. This was confirmed by the resistant reaction of ‘Salinas’. The resistant reaction of ‘Banchu Red Fire’ was unexpected, as it was reported susceptible to race 1 in Japan (Fujinaga et al.,

2003). These results suggest the possibility of a unique race in Salinas Valley, or mis-identified/mis-labeled seed for this FW race differential.

Mean disease reaction of the FW-susceptible parent, ‘Autumn Gold’, in the two tests was 1.8, which meant that on average it exhibited slight-moderate stunting. ‘King Louie’ was highly resistant in both tests (Table 3) The six F5 selections were comparable to ‘King Louie’ for reaction to FW. Plants were sampled for examination of root symptoms to confirm presence of FW in the tests. ‘Patriot’ roots consistently exhibited internal discoloration typical of FW infection (Figure 2, left). In contrast, the FW-resistant lines had healthy roots, typified by line 15333 (Figure 2, right).

Table 2. Mean disease reactions of lettuce Fusarium wilt race differentials and iceberg lettuce ‘Salinas’ in two naturally-infected commercial field tests in Salinas Valley, Summer 2018.^z

Entry	Test 1		Test 2		Combined
	Mean	n	Mean	n	
Patriot	3.0	169	2.6	198	2.8
Costa Rica No. 4	1.0	21	1.0	208	1.0
Banchu Red Fire	1.1	174	1.0	227	1.0
Salinas	1.2	203	1.2	210	1.2

^zRated on a 1 to 4 scale of foliar symptoms, where 1 = no apparent disease symptoms, 2 = slight-moderate stunting, 3 = severe stunting, 4 = senesced, i.e., dead.

Table 3. Mean disease reactions of six F₅ lettuce Fusarium wilt-resistant breeding lines and their parents in two naturally-infected commercial field tests in Salinas Valley, Summer 2018.^z

Entry	Test 1		Test 2		Combined
	Mean	n	Mean	n	
Autumn Gold (susceptible)	2.1	151	1.5	180	1.8
King Louie (resistant)	1.0	127	1.0	103	1.0
15631	1.0	126	1.0	129	1.0
15632	1.0	112	1.0	131	1.0
15633	1.0	38	1.0	58	1.0
15634	1.0	87	1.1	78	1.0
15669	1.1	107	1.0	142	1.0
15670	1.0	81	1.1	70	1.1

^zRated on a 1 to 4 scale of foliar symptoms, where 1 = no apparent disease symptoms, 2 = slight-moderate stunting, 3 = severe stunting, 4 = senesced, i.e., dead.



Figure 2. Roots of Fusarium wilt-susceptible ‘Patriot’ (left) and resistant breeding line 15633 (right) in a naturally infected commercial field test in Salinas Valley, Summer 2018.

The lines were selected for FW resistance in controlled-inoculation greenhouse tests and tested in commercial field tests in Yuma to confirm their greenhouse test disease reactions and select for horticultural quality, i.e., ‘Salinas’, type for the next round of selection for FW resistance in greenhouse tests. The six breeding lines were selected from a single F4 family, and may be considered sib-lines. The lines are not uniform for iceberg-type head formation (Figure 3) but are uniform for the ‘Salinas’ leaf type and coloration (Ryder, 1979).

The six FW-resistant breeding lines were approved for release by USDA-ARS (see attachment). Seeds (F6) of the six lines are being produced in a greenhouse for distribution later in 2019. The lines were bred for adaptation to the early-Fall season of Yuma. ‘Autumn Gold’. Their FW-susceptible parent is a Salinas type, iceberg lettuce developed for early Fall production in the lower deserts of Arizona and California (Ryder et al., 1991). It exhibited moderate resistance to FW in the Salinas Valley test as compared with ‘Patriot’. ‘King Louie’, the FW-resistant parent, is a commercially developed romaine lettuce that exhibited high-level resistance to FW in commercial field tests in Yuma. (Matheron et al., 2005).



Figure 3. Variation for head formation in *Fusarium* wilt- resistant breeding line 15631.

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IMPATIENS NECROTIC SPOT VIRUS (with *W. Wintermantel*)

The thrips-vectored orthotospovirus, *Impatiens necrotic spot virus* (INSV), causes economic damage due to spotted wilt disease on lettuce in coastal California. INSV has become more widespread and severe in the Salinas Valley in recent years, associated with large populations of its thrips vector. Initial symptoms appear as small brown lesions or spots that expand into larger necrotic sections on infected leaves. Stunted, necrotic, or otherwise symptomatic plants are not marketable; high disease incidence results, therefore, in substantial economic losses to growers. The wide range of host species that can be infected by INSV complicates disease control. Therefore, the best strategy for control is the use of INSV-resistant cultivars, for which there is evidence. In previous research efforts, a large collection of germplasm was evaluated for INSV resistance and identified as susceptible, intermediate, or partially resistant. Many crosses were made between susceptible and resistant lines. Our objectives for 2018-2019 were to establish a viruliferous thrips colony for germplasm evaluation and to screen progeny of crosses for resistance to INSV in greenhouse experiments. First, a source of western flower thrips was identified at the USDA-ARS station in Salinas and a colony was established in a dedicated greenhouse containing INSV-infected lettuce and *Emilia sonchifolia* plants. Plants were monitored for thrips damage and INSV symptom development.

Once a stable INSV-infected thrips population was established, we began evaluating F1 progeny from crosses of resistant and susceptible lines. Previous research identified the cultivar Eruption as having partial resistance and Conquistador as susceptible. These lines were crossed, giving rise to the F1 population SMF1-050. Approximately 20 seeds of Eruption, Conquistador, and *E. sonchifolia* and 40 seeds of SMF1-050 were planted into 72-cell plug trays in a standard greenhouse potting soil (Premium Growers Mix, Sun Land Garden Products, Watsonville, California), and maintained in a cool growth chamber at 10° C with no light for two days to improve uniformity of germination. Seedlings were then transferred to a greenhouse until two true leaves are fully emerged, then transplanted to 10 × 10 cm pots containing potting soil. At the 3-4 true leaf stage, 10 seedlings of Eruption, Conquistador, and *E. sonchifolia* and 39 seedlings of SMF1-050 were rub-inoculated with INSV. Inoculum was prepared by adding 1-2 g of INSV infected *E. sonchifolia* (L.) DC. leaf tissue that had been inoculated with INSV by thrips transmission, suspended in approximately 3 ml of mechanical inoculation buffer (100 mM sodium phosphate buffer, pH 7.0 containing 0.2 % sodium sulfite) and 0.05 g Celite as an abrasive. It is critical that inoculum be derived from a source infected by thrips transmission of virus, because infectivity decreases greatly with successive mechanical (rub) inoculations. Plants were rinsed with water following inoculation to remove remaining Celite. Leaves used for transmission experiments were tested prior to inoculation using immunostrips (Agdia, Elhart, IN) to confirm infection with INSV, and to verify inoculum source plants were not infected with *Tomato spotted wilt virus* (TSWV). Ten seedlings each of Conquistador and *E. sonchifolia* were left un-inoculated. After four weeks, plants were given a final visual evaluation of INSV (presence or absence of symptoms) and thrips damage (rated 0-5) symptoms and 0.5 g leaf tissue was taken from each plant for determination of virus infection and titer by enzyme-linked immunosorbent assay (ELISA) using the Agdia INSV ELISA detection kit per manufacturer's recommendations. Tissue used for the tests originated from leaves that were younger than those mechanically inoculated with INSV to confirm virus replication and systemic movement in the

lettuce plant. Each ELISA plate included three positive and three negative controls. Only the central 60 wells of the 96-well plate were used for tests to ensure high uniformity of conditions. Positive controls were *E. sonchifolia* plants previously confirmed to be infected by INSV. Negative controls were healthy lettuce plants. Samples were considered to be positive if the ELISA absorbance readings were 2.5x higher than the mean of negative controls.

Of the *E. sonchifolia* plants tested, nine were positive and one was negative for INSV symptoms, yet all plants tested positive for the virus using ELISA (Table 4). The susceptible cultivar Conquistador had nine plants positive and one negative for INSV symptoms. These ELISA results matched with INSV symptoms and showed seven positive and one negative ELISA (two plants were dead from INSV at time of ELISA). The partially resistant parent cultivar Eruption only had one plant positive and nine negative for INSV symptoms. ELISA showed three positive and seven negative for the virus. When the F1 progeny were tested (SMF1-050) of the Conquistador x Eruption cross, 23 plants were negative for INSV symptoms, yet of these, 20 were positive for INSV. The remaining 16 plants were unclear if symptoms were due to manual inoculation or INSV infection. The discrepancies between visible INSV symptoms and ELISA results are likely due to the sensitivity of the ELISA over the naked eye. The segregation of ELISA values for the F1 progeny is unexpected. If resistance from Eruption was dominant, we would expect to see all progeny resistant (negative ELISA) and if it was recessive, we would expect all susceptible (positive ELISA). Instead we see a 3 susceptible to 1 resistant ratio, as confirmed by Chi-squared analysis. This could be due to resistance in Eruption segregating, but it does not fit the then expected 1:1 ratio. We continue to evaluate additional F1 progenies from different crosses.

Table 4. Number (n) of plants positive (+) and negative (-) for symptoms of infection and ELISA virus titer of *Impatiens necrotic spot virus* and thrips damage (0-5 scale) of *Emilia sonchifolia*, Conquistador, Eruption, and F1 progeny after manual inoculation and viruliferous thrips.

Entry	Description	n	INSV	ELISA	Thrips
<i>E. sonchifolia</i>	Susceptible	10	1-, 9+	10+	3.7
Conquistador	Susceptible	10	1-, 9+	1-, 7+, 2 dead	4.2
Eruption	Resistant	10	9-, 1+	7-, 3+	3.7
Conquistador x Eruption	F ₁ progeny	39	23-, 16 unclear	12-, 27+	2.6

BACTERIAL LEAF SPOT (in collaboration with *J. Sthapit*)

Bacterial leaf spot (BLS) disease of lettuce is caused by bacteria *Xanthomonas campestris* pv. *vitians* (*Xcv*). In baby leaf lettuce, seeds are planted in extremely high density in the field, which provides a perfect habitat for the development of BLS. It can cause significant economic damage, mainly during cool and wet weather conditions. Cultivating resistant cultivars is the most sustainable and reliable strategy to manage BLS. Identification of resistant germplasm will provide essential information and facilitate in breeding for BLS resistance.

Four hundred and ninety-five lettuce accessions were evaluated for resistance to BLS. Seeds were planted in plug trays with 31 rows of 11 cells with dimension of each cell being 19 x 19 x 60 mm. Each accession was planted in three wells. For germination, the planted trays were put in growth chambers in dark at 10°C for 2 days. Then growth chamber program was set to 16 hours of light at 20°C. Three weeks after planting, the plants were moved to greenhouse and spray inoculated with *Xcv* strain BS2861. Inoculated plants were kept wet with the use of programmed water misters to facilitate disease development. Disease severity was evaluated at three weeks after inoculation using 0 to 5 rating scale where 0 = no disease, 1 = little disease, a few lesions of < 3 mm in size, 2 = individual disease lesions > 3 mm or more than 10 lesions covering < 10% of leaf area, 3 = large coalesced lesions covering 10 to 20% of leaf area, 4 = lesions covering 20 to 50% of leaf area, and 5 = lesions covering greater than 50% of leaf area.

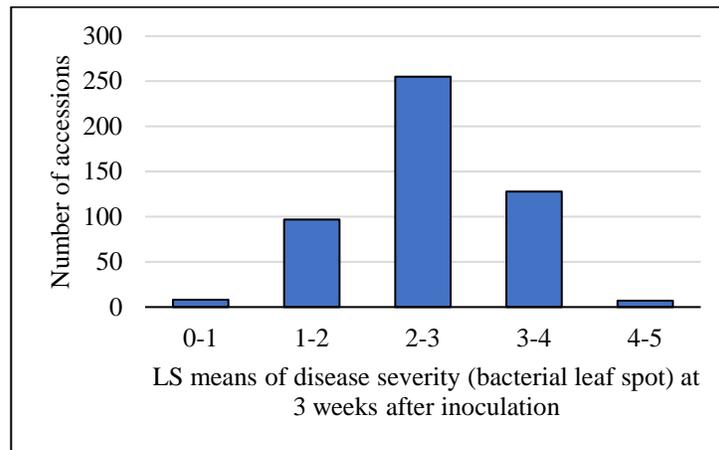


Figure 4. Distribution of LS means of disease severity ratings for bacterial leaf spot in 495 lettuce accessions at three weeks after inoculation.

Figure 4 shows the distribution of least square means of disease severity ratings among 495 lettuce accessions. The five accessions with lowest BLS disease severity to BS2861 were PI 226514, ARM09-161-10-1-4, RH10-0262, PI 278070 1-COS-8, and SalVal-111. Five accessions with highest disease severity were PI 358040, PI 634687, PI 179295, PI 167128, and PI 251246. All the accessions will be reevaluated in future experiments with three bacterial strains in at least three experiments for each strain to access variation in reaction to BLS among different lettuce genotypes.

LEAFMINER

The predominant species of leafminers in central California is *Liriomyza langei*. They have a wide host range including broccoli, cauliflower, celery, lettuce, melons, spinach, tomato, and many weeds. Damages caused by adult sting and larval mining of leaves reduce photosynthetic capacity and product quality, render lettuce leaves unmarketable, and provide an entrance for disease organisms. Chemical control is not long lasting, and it is well documented that leafminers can develop a high degree of resistance to insecticides. The most economical means to control leafminers is through the use of plant resistance.

We made crosses to transfer leafminer resistance from wild species into iceberg and mixed lettuce types. BC₁F₂ to BC₁F₆ plants from these crosses were selected in the field for horticultural traits and resistance to leafminer, and were backcrossed if necessary to restore horticultural types. We also continued to make crosses to combine leafminer resistance with resistances to other diseases and insects for multiple-resistance. Crosses were also made among resistant sources in an effort to elevate the level of resistance to leafminers.

F₂ to F₆ progeny plants from crosses between leafminer resistant source (PI 169513, Red Grenoble, Merlot, Lolla Rossa, Bibb, and Tom Thumb) and good horticultural types (Salinas, Salinas 88, Tiber, Prizehead, and Lobjoits) were selected in the field for leafminer and multiple resistances, and some of them were backcrossed to restore horticultural traits. Eighteen promising F₇ to F₁₁ breeding lines of green leaf, red leaf, and romaine lettuces were trialed at Spence Farm in Salinas from June to September 2018 with four replications, along with commercial cultivars and resistant controls. All breeding lines had significantly lower leafminer sting density than commercial cultivars and resistant controls, and the plant weight, core length, and tipburn of the breeding lines were generally similar to or better than commercial cultivars (Tables 5-7). Many of these breeding lines also have the *cor* resistant gene, so they are resistant to corky root disease as well (Tables 5-7). Some green leaf lettuce breeding lines showed moderate to high levels of resistance to downy mildew. A breeding line, MU17-340, had a downy mildew disease rating similar to the resistant control ‘Grand Rapids’ (Table 5). These breeding lines will be evaluated in the field again in 2019 to confirm the results.

Table 5. Mean values of leafminer sting density and head characteristics of green leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2018.

Genotype ^z	Stings/cm ² . ^y	Plant Wt. (g)	Core length (cm)	Downy mildew ^x
Waldmann’s Green	2.9 A	458.7 B	7.6 A	3.0 B
Grand Rapids	2.2 B	290.7 C	4.9 E	1.5 D
Two Star	2.1 B	598.5 A	6.4 BC	4.0 A
Shining Star	1.7 C	490.5 B	6.9 B	4.0 A
MU17-337 (<i>cor</i>)	0.4 D	506.7 B	5.4 DE	1.8 D
MU17-340 (<i>cor</i>)	0.4 D	635.7 A	6.5 B	1.5 D
MU17-348 (<i>cor</i>)	0.2 D	611.3 A	6.2 BC	2.0 CD
MU17-352 (<i>cor</i>)	0.2 D	610.8 A	5.7 CD	2.5 BC

^z Some breeding lines have the *cor* gene and are resistant to corky root. ^y Means in the same column followed by different letters indicate significant differences at $P < 0.05$. ^x On a scale of 0 – 5 (0, no lesion; 5, large lesions covering nearly 100% of the exposed leaf surface).

Table 6. Mean values of leafminer sting density and head characteristics of red leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2018.

Genotype	Stings/cm ^{2,z}	Plant Wt. (g)	Core length (cm)	Tipburn ^y
Big Red	4.5 A	466.5 AB	8.8 AB	0.6 A
Prizehead	3.5 B	492.7 AB	6.2 CD	0.0 A
Red Fox	3.3 B	528.8 A	7.9 ABC	0.0 A
Lolla Rossa	2.0 C	200.0 C	5.1 D	0.0 A
Red Hot	1.9 C	188.2 C	5.4 D	0.0 A
Merlot	1.9 C	231.5 C	6.1 CD	0.0 A
MU17-344	0.7 D	498.0 AB	9.3 A	0.0 A
MU17-351	0.7 D	425.0 B	6.9 BCD	0.0 A

^z Means in the same column followed by different letters are significantly different at $P < 0.05$.

^y Number of leaves with tipburn in a head.

Table 7. Mean values of leafminer sting density and head characteristics of romaine lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2018.

Genotype ^z	Stings/cm ^{2,y}	Plant Wt. (g)	Core length (cm)	Tipburn ^x
Green Forest	4.6 A	969.9 B	9.7 A	0.0 B
Valmaine	4.2 AB	890.0 B	7.8 C	0.3 AB
Heart's Delight	3.8 AB	1,031.5 AB	9.5 A	0.4 AB
Parris Island Cos	3.8 AB	877.0 B	7.6 CD	0.1 B
Clemente	3.2 B	1,190.9 A	9.3 AB	0.6 AB
MU17-362 (<i>cor</i>)	0.4 C	857.2 B	7.3 CDE	0.0 B
MU17-534 (<i>cor</i>)	0.2 C	890.3 B	6.2 E	0.6 AB
MU17-536 (<i>cor</i>)	0.2 C	873.8 B	6.6 DE	0.0 B
MU17-361 (<i>cor</i>)	0.2 C	861.8 B	8.2 BC	0.9 A
MU17-355	0.2 C	1,008.0 B	7.7 CD	0.0 B

^z Some breeding lines have the *cor* gene and are resistant to corky root. ^y Means in the same column followed by different letters indicate significant differences at $P < 0.05$. ^x Number of leaves with tipburn in a head.

LETTUCE APHID (McCreight, Liu)

This project has been working with in *Lactuca serriola* PI 491093 which exhibited an intermediate level of resistance to biotype Nr:0 in greenhouse tests as compared with aphid resistant genotype, e.g., 'Dynamite' (Arend et al., 1999), but which was nearly aphid-free in field tests. Progress was made in evaluating backcross (BC2) progenies from the cross of PI 491093 x Salinas. PI 491093, but results may have been confounded by the presence of Nr:1 in the lettuce aphid colony.

Lettuce aphid strain Nr:1 emerged in Europe in 2007 (ten Broeke et al., 2013). This biotype is virulent on cultivars with resistance to Nr:0. Nr:1 has been reported in Australia (https://www.rijkzwaan.com.au/sites/default/files/lettuce_all_nasonovia_nr1_20180130_a4.pdf ; <https://ausveg.com.au/articles/resistance-breaking-nasonovia-biotype-confirmed-australia/>). There were reports of lettuce aphids on Nr:0-resistant lettuce cultivars in Salinas Valley. Tests are under way to document the presence Nr:1 in the lettuce aphid colony.

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TIPBURN (in collaboration with *R. Michelmore, R. Hayes, S. Jenni, L. Bertier, M. Truco, and M. Macias-Gonzalez*)

Tipburn is a physiological disorder characterized by necrotic lesions on leaf margins and is triggered by environmental factors that are not completely understood. In commercial lettuce, even low incidence of tipburn renders the heads unsalable. In addition, tipburn provides entry points for postharvest storage decay organisms that reduce shelf life and culinary quality. We conducted research to elucidate the genetic control of resistance to tipburn in lettuce using seven lettuce populations, derived from different combinations of parents that differed in their susceptibility to tipburn; testing them in multiple environments and years to identify quantitative trait loci (QTL) for tipburn. Core height, head firmness, head closure, leaf crinkliness, plant fresh weight, and leaf savoy were also analyzed to investigate whether QTL for these morphological traits collocated with QTL for tipburn, which would be indicative of pleiotropic effects. Twenty-three major, intermediate, and minor unique QTL for tipburn were identified in one or more populations scattered throughout the genome. Two major QTL for tipburn incidence were identified in linkage groups (LG) 1 and 5 (Figure 5), which determined up to 45 and 66% of the phenotypic variance. The major QTL in LG 1 collocated with the head firmness QTL. The major QTL in LG 5 collocated with the QTL for core height, leaf crinkliness, and head firmness. Further research is needed to determine whether these associations are due to pleiotropic effects of the same gene or if the genes determining these traits are tightly linked. The beneficial alleles at the QTL in LG 1 and 5 are present in the widely grown cultivar Salinas, the genotype sequenced for the reference genome assembly. These QTL are good targets to identify genes controlling tipburn reaction as well as regions for marker-assisted selection to improve resistance to tipburn in lettuce.

Table 8. Tipburn incidence, number infected with *Impatiens necrotic spot virus*, maturity, number of marketable heads, head closure, head height, core length, bottom width and top width of 215 F6:7 derived from a cross of Green Towers (low incidence of tipburn, romaine) and Salinas (low incidence of tipburn, iceberg).

Lines tested	Previous No.	No. tipburn	No. INSV	Maturity	No. marketable	Head closure	Height	Bottom	Top
Green Towers	Romaine	6.44	4.67	3.58	8.83	2.42	29.13	14.60	28.73
Salinas	Iceberg	3.50	3.14	4.21	15.71	iceberg	15.07	7.17	8.02
RH15-0968-0990	RH13-0018	3.30	1.36	4.19	9.32	3.61	30.02	14.93	27.90
RH15-1136-1145	RH13-0022	3.33	2.33	3.80	8.13	2.77	33.40	15.97	33.09
RH15-1059-1069	RH13-0020	3.67	2.27	4.79	8.21	4.52	37.53	13.47	22.69
RH15-0924-0950	RH13-0017	3.88	2.07	4.23	11.52	3.65	31.06	13.96	28.17
RH15-1007-1029	RH13-0019	4.30	1.48	4.00	9.23	3.67	30.02	14.80	27.69
RH15-0764-0779	RH13-0013	4.98	4.67	2.86	11.48	2.03	29.46	15.83	26.89
RH15-0721-0743	RH13-0012	5.09	4.84	3.07	10.45	1.93	28.61	15.63	26.13
RH15-1093-1105	RH13-0021	5.18	2.21	3.97	8.26	3.31	33.17	14.87	14.87
RH15-0876-0902	RH13-0016	5.57	4.25	2.96	10.12	1.77	8.11	15.18	27.23
RH15-0840-0861	RH13-0015	6.06	4.70	3.32	12.18	2.16	8.59	14.60	26.43
RH15-0800-0819	RH13-0014	6.08	4.73	3.16	11.22	2.11	8.96	14.63	26.37

POSTHARVEST DETERIORATION OF FRESH-CUT LETTUCE (in collaboration with *H. Peng, J. Sthapit, and R. Micheltore*)

To study the genotypic and phenotypic variation in shelf life, over 500 lettuce accessions were tested in replicated field trial at locations (Marina, Gonzales, Salinas, CA) and different planting seasons. Lettuce heads were harvested and processed into three salad bags (340 g each) per plot, and stored at 4°C for evaluation. Salad bags were evaluated on weekly intervals for at least six weeks using a 0 (no deterioration) to 10 (100% deterioration) scale. Least square means (LS means) of the deterioration scores were calculated for each accession for evaluation every week, which was then used to calculate area under deterioration progress stairs (*AUDePS*) for all the accessions for each experiment. As all evaluations were performed at regular intervals and at every time unit (weekly), *AUDePS* was calculated as follows: $AUDePS = \bar{y} \times n$, where \bar{y} is the arithmetic mean of all observations and n is the total number of observations.

Deterioration rate (DR) of fresh-cut lettuce are affected by many factors such as physiological status, procession method, wounding (Figure 6), storage condition, atmosphere, and hormone level. Information of those factors highly associated with decay development can give indicate the critical pathway in which *qSL4* (the gene conferring the rate of deterioration) might function and thus could help narrow down the pool of candidate genes.

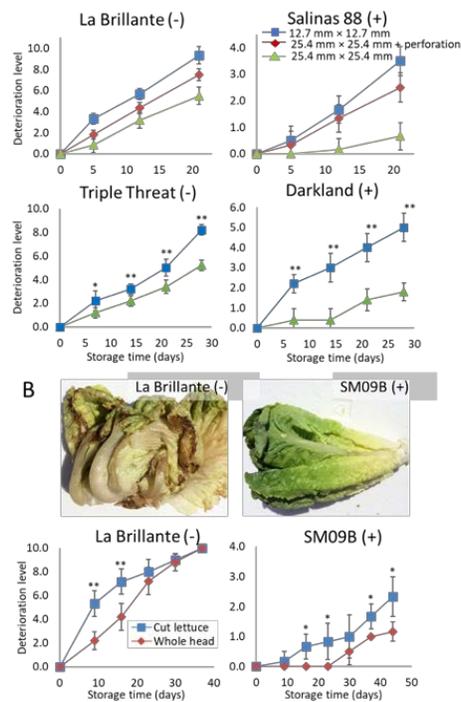


Figure 6. Impact of physical wounding on deterioration rate of fresh-cut lettuce in modified atmosphere packaging (MAP). A, Deterioration rate of cut lettuce with additional wounding. B, Deterioration rate of whole heads and cut lettuce in MAP. Photos of whole heads were taken after they were stored in MAP for 16 days. Data presented are mean values of deterioration rates of at least five replicates and the error bars represent the 95% confidence interval (CI95). Multiple comparison of sample values was done by Tukey-Kramer test after one-way ANOVA

analysis. Symbols * and ** indicate significance $p < 0.05$ and $p < 0.01$, respectively. Slowly and rapidly deteriorating cultivars are labeled with (+) and (-), respectively.

Samples of small size had more intensive browning than larger samples, indicating a possibly higher O_2 content in bags with less tissue. Samples at large packing size showed faster deterioration in both rapidly and slowly deteriorating cultivars (Figure 7).

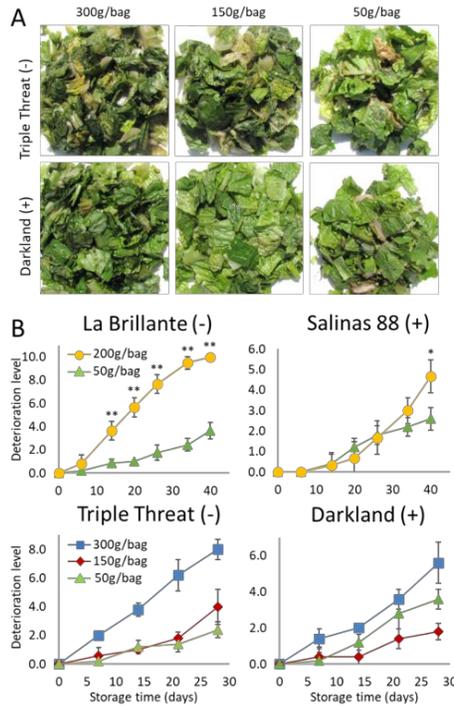


Figure 7. Effect of sample size on deterioration rate of fresh-cut lettuce in modified atmosphere packaging (MAP). A, Appearance of cut lettuce packed at different size in MAP for 28 days. Slowly and rapidly deteriorating cultivars are labeled with (+) and (-), respectively. B, Deterioration rate of cut lettuce packed at different size in MAP. Data presented are mean values of deterioration rates of at least five replicates and the error bars represent the 95% confidence interval (CI95). Multiple comparison of sample values was done by Tukey-Kramer test after one-way ANOVA analysis (see Supplemental Table S1 for p values of each comparison). Symbols * and ** indicate significance $p < 0.05$ and $p < 0.01$, respectively.

To further understand the molecular basis of fast deterioration, RNAseq was applied to compare gene expression profiles of rapidly (La Brillante, LB) and slowly (Salinas 88, S88) deteriorating cultivars during storage. When tissues started to deteriorate, more genes were up-regulated in rapidly deteriorating cultivar than in slowly deteriorating cultivar, indicating more cellular activity in the fast deteriorating samples. Differentially expressed (DE) genes majorly involved in cellular process (16%), followed by metabolic process (13%) and stimulus responses (11%).

MOLECULAR MARKER DEVELOPMENT

VERTICILLIUM WILT, RACE 1 (with *P. Inderbitzin*, *K. Subbarao*, *R. Michelmore*, *M. Christopoulou*, *D. Lavelle*, and *S. Reyes-Chin Wo*)

The Salinas and Pajaro Valleys of coastal central California are among the most important lettuce-producing regions in the United States. One of the top disease concerns for lettuce in the area is Verticillium wilt caused by the fungus *Verticillium dahliae*, which is a soilborne pathogen with a wide host range that also includes artichoke, cotton, eggplant, hops, potato, sunflower, tobacco, and tomato. Resistance of lettuce against *V. dahliae* race 1 was previously mapped to the single dominant *Verticillium resistance 1* (*Vr1*) locus. We used genome sequencing and/or PCR screening along with pathogenicity assays of 151 accessions of lettuce to investigate allelic diversity and its relationship to race 1 resistance in lettuce. This approach identified a total of four *Ve* genes; *LsVe1*, *LsVe2*, *LsVe3*, and *LsVe4*. The majority of accessions, however, contained a combination of only three of these *LsVe* genes clustered on chromosomal linkage group 9 (Figure 8). A single allele, *LsVe1L*, was present in all resistant accessions and absent in all susceptible accessions. This allele can be used as a molecular marker for *V. dahliae* race 1 resistance in lettuce. A PCR assay for rapid detection of race 1 resistance in lettuce was designed based on nucleotide polymorphisms. Application of this assay allows identification of resistant genotypes in early stages of plant development or at a seed-level without time- and labor-intensive testing in the field. The manuscript with detailed description of results has been submitted to the scientific journal and is expected to be published in 2019.

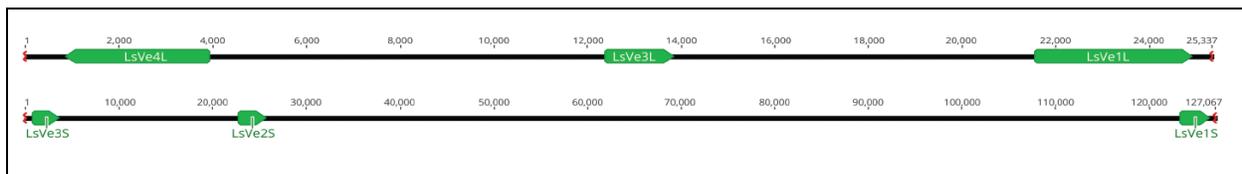


Figure 8. Partial scaffolds of lettuce cultivars La Brillante (resistant to *V. dahliae*, race 1) and Salinas (susceptible to *V. dahliae*, race 1) showing positions of *LsVe* genes.

RECENT (2018 - 2019) PUBLICATIONS RELEVANT TO THIS PROJECT

Fletcher, K, Klosterman, S, Derevnina, L, Martin, F, Bertier, L, Koike, S, Reyes-Chin-Wo, S, Mou, B, and Michelmore, R (2018) Comparative genomics of downy mildews reveals potential adaptations to biotrophy. *BMC Genomics* 19:851. <https://doi.org/10.1186/s12864-018-5214-8>

Hayes RJ, Sandoya G, Mou B, Simko I, Subbarao KV (2018) Release of three iceberg lettuce populations with combined resistance to two soilborne diseases. *HortScience* 53:247-250. <https://doi.org/10.21273/HORTSCI12559-17>

Macias-Gonzalez M, Truco MJ, Bertier LD, Jenni S, Simko I, Hayes RJ, Michelmore RW (in press) Genetic architecture of tipburn resistance in lettuce. *Theoretical and Applied Genetics*

Mamo BE, Hayes RJ, Truco MJ, Puri KD, Michelmore RW, Subbarao KV, Simko I (in press) The genetics of resistance to lettuce drop (*Sclerotinia* spp.) in lettuce in a recombinant inbred line population from Reine des Glaces x Eruption. *Theoretical and Applied Genetics*

Simko I, Hayes RJ (2018) Accuracy, reliability, and timing of visual evaluations of decay in fresh-cut lettuce. *PLoS One* 13: e0194635

Simko I, Hayes RJ, Truco MJ, Michelmore RW, Antonise R, Massoudi M (2018) Molecular markers reliably predicts post-harvest deterioration of fresh-cut lettuce in modified atmosphere packaging. *Horticulture Research* 5:21

Simko I, Richardson CE, Wintermantel WM (2018) Variation within *Lactuca* spp. for resistance to Impatiens necrotic spot virus. *Plant Disease* 102:341-348

Sytar O, Zivcak M, Bruckova K, Brestic M, Hemmerich I, Rauch C, Simko I (2018) Shift in accumulation of flavonoids and phenolic acids in lettuce attributable to changes in ultraviolet radiation and temperature. *Scientia Horticulturae* 239:193-204

Xu, C and Mou, B (2018) Chitosan as soil amendments affects lettuce growth, photochemical efficiency and gas exchange. *HortTechnology* 28: 476-480. doi: <https://doi.org/10.21273/HORTTECH04032-18>

Attachment: Germplasm Release Notice

U.S. Department of Agriculture, Agricultural Research Service-approved germplasm release notice for six Fusarium wilt-resistant lettuce breeding lines.



United States Department of Agriculture

Research, Education, and Economics
Agricultural Research Service

RELEASE OF FUSARIUM WILT-RESISTANT LETTUCE BREEDING LINES 15631, 15632, 15633, 15634, 15669, 15670

The Agricultural Research Service, United States Department of Agriculture (USDA), announces the release of Fusarium wilt-resistant lettuce breeding lines. Lettuce is the most popular leafy green vegetable in the United States. It is produced year-round in California and Arizona, shifting from central, coastal California (Salinas, Pajaro and Santa Maria Valleys) in the summer months through the Central Valley of California to the lower desert areas of California (Coachella, Imperial and Palo Verde Valleys) and Arizona (Yuma area) in the winter months. The breeding lines were developed at the United States Agricultural Research Station, Crop Improvement and Protection Research Unit, Salinas, California by James D. McCreight.

Fusarium wilt has become a major disease of iceberg lettuce in the early Fall season in Arizona and has recently emerged in areas of the Pajaro and Salinas Valleys. The disease first appeared in the Huron production area of the Central Valley in 1991, where growers were able to shift production to disease-free fields. Fusarium wilt emerged in Yuma in Fall 2001 and has continued to spread to throughout the region. The disease was mostly associated with hot production areas, such as late-Summer planting dates in Huron (August) and the early-Fall planting dates (September) in Yuma. The pathogen was known in the relatively cooler Pajaro Valley in 2004 but was controlled through rotation with strawberry plantings that were always fumigated with a combination of methyl bromide and chloropicrin for soilborne disease, insect, and weed control. Fusarium wilt has more recently emerged in the neighboring Salinas Valley and may occur with Verticillium wilt incited by the soilborne fungus, *Verticillium dahliae*.

The breeding lines were derived from a cross of 'King Louie' with 'Autumn Gold' using standard procedures for cross-pollinating lettuce in a greenhouse; the F2 was produced in the same greenhouse at Salinas. 'King Louie' is a Romaine type lettuce that was found to be highly resistant to Fusarium wilt in naturally-infected field tests in Yuma. 'Autumn Gold' is a Fusarium wilt-susceptible, iceberg type lettuce adapted to the early-Fall planting (September 1 to 20) time in Imperial Valley and Yuma. Fusarium wilt-resistant F2 segregants were selected in controlled inoculation greenhouse tests and saved to produce the F3 generation. The F3 families were evaluated in controlled inoculation greenhouse tests and their reactions confirmed in naturally-infected field tests in a commercial field in Yuma. Selected F4 families were evaluated for Fusarium wilt resistance in field tests in Yuma. Selected F5 families were field tested for disease reaction and plant type in Salinas Valley. The released germplasm is a group of F5 and F6 families resistant to Fusarium wilt and varying for plant type that is similar to iceberg; a few lines exhibit leaves with red coloration (anthocyanin).

Office of the Administrator
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1400 Independence Avenue, SW.
Washington, D.C. 20250
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Attachment: Germplasm Release Notice

U.S. Department of Agriculture, Agricultural Research Service-approved germplasm release notice for six Fusarium wilt-resistant lettuce breeding lines.

The breeding lines are freely available to lettuce breeders and seedsmen for development of Fusarium wilt resistant lettuce. Written seed requests should be sent to Dr. Jim McCreight, USDA-ARS, 1636 E. Alisal St., Salinas, CA 93905; jim.mccreight@usda.gov.

ARS GIVES NO WARRANTIES OR GUARANTEES, EXPRESSED OR IMPLIED, FOR THE MATERIAL, INCLUDING MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.



Acting Deputy Administrator, Crop Production and Protection
Agricultural Research Service, U.S. Department of Agriculture

2/28/19

Date

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U.S. Department of Agriculture, Agricultural Research Service-approved germplasm release notice for six Fusarium wilt-resistant lettuce breeding lines.