PROJECT REPORT
CALIFORNIA LETTUCE RESEARCH BOARD

For the period
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PROJECT TITLE: Lettuce Breeding, USDA-ARS

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ABSTRACT:

In the 2014-2015 period, major efforts targeted resistance to lettuce big vein disease, lettuce drop caused by *Sclerotinia* species, Verticillium wilt, Fusarium wilt, bacterial leaf spot, corky root, downy mildew, leafminer, lettuce aphid, tipburn, shelf-life of salad-cut lettuce, survival of human enteric pathogens on lettuce, and multiple disease resistance. In all programs, horticultural traits, adaptation, and resistance to tipburn are essential.

OBJECTIVES:

Development of new landmark lettuce cultivars and breeding lines with improved disease resistance, insect resistance, tolerance to heat and cold stress, uniform growth and maturity, horticultural quality, postharvest quality, and adaptation to specific lettuce districts and seasons.

PROCEDURES AND RESULTS:

A. Cultivar and advanced breeding line development

1. Disease resistances

a. Lettuce big-vein disease (with W. Wintermantel)

Big-vein is a soil borne viral disease of lettuce. A high level of partial resistance to big-vein disease is available in the butterhead cultivar Margarita; we are introgressing this resistance into iceberg breeding lines. In a February planted field experiment in Salinas, CA, we selected and developed nine BCF_5 and 16 BCF_3 iceberg breeding lines with reduced incidence of big-vein disease compared to cv. Sniper.

Quantitative trait loci for big-vein resistance were identified in a recombinant inbred line population from Salinas 88 × PI 251246. We had planned to repeat these experiments in 2014, but were unable to due to the unavailability of big-vein infested field space at the USDA research station.

b. Lettuce drop (with K. Subbarao)

Lettuce drop is a near ubiquitous soil borne disease of lettuce caused by *Sclerotinia minor* and *S. sclerotiorum*. We initiated a single seed descent breeding program for lettuce drop resistance. Overall, this method is expected to increase selection efficiency and have a shorter breeding cycle time compared to previous approaches. We inbred 163 lines from Reine des Glaces × Eruption to the F_6 generation. These RILs will be used for genetic experiments. In two field experiments, we evaluated 82 F_6 iceberg lines derived from a complex pedigree involving Great Lakes 54, Holborn Standard, and modern iceberg cultivars. Thirty seven were selected for reevaluation in 2015. Three inbred romaine lines were selected from Darkland × RH07-0731-4-3 for having better resistance than Darkland. Seed of these three lines were increased for additional testing. Crosses were made among diverse sources of resistance to develop 12 new breeding populations.

The red Latin type cultivar Eruption has demonstrated resistance to sclerotial infection by *Sclerotinia minor* and *S. sclerotiorum* and to ascospores from *S. sclerotiorum*. The resistance appears to be independent of plant morphology, which means that this resistance can be introgressed into diverse market types. We are currently introgressing resistance from the cultivar Eruption into romaine breeding lines. Several inbred romaine breeding lines from Hearts Delight × Eruption have routinely demonstrated significantly lower mortality than commercial romaine cultivars, but were poorly characterized for yield and horticultural quality. Forty-eight inbred lines were evaluated for yield, head weight, core height, and resistance in two unreplicated experiments in grower fields. Eight lines were selected for additional testing. These lines generally have shorter cores than commercial cultivars, but have heads that are approximately 50 grams lighter than commercial cultivars. The shelf-life of fresh-cut salad of the eight selected lines was similar or slightly inferior to Green Towers. The arrival quality of whole heads has not been tested. As in previous experiments, all the breeding lines had better resistance than modern commercial cultivars.

Diverse sources of resistance are being used in a modified pedigree breeding approach to develop romaine and leaf germplasm with resistance to lettuce drop. In this breeding scheme, spring and summer planted infested field experiments are conducted to identify families and breeding lines with improved resistance. A non-infested field experiment is conducted concurrently; single plants with improved horticultural characters are taken from only the resistant families. Single plant selections are allowed to self-pollinate to develop seed of the next generation for use in subsequent experiments. Using this method we have developed resistant romaine and leaf germplasm. *Romaine breeding:* The Hearts Delight × Eruption breeding lines RH09-0488 and RH09-0519 were used in crosses to develop new populations for breeding. Eighteen, 39, and 14 F_3 families from RH09-0488 × RH09-0519, RH09-0488 × Green Towers, and RH09-0488 × PI 226641, respectively, were evaluated for resistance in a replicated field experiment. PI 226641 is a partially resistant romaine accession. The RH09-0488 × RH09-0519 families did not appear to segregate for resistance, and all were selected for retesting. Nineteen families from RH09-0488 × Green Towers and four families from RH09-0488 × PI 226641 were selected for reevaluation. *Leaf breeding:* In a replicated field experiment, we identified fourteen leaf type F_4 families from Two Star × Eruption and three leaf type F_4 families from PI 178924 × Red Fox with better resistance than Two Star and Red Fox. PI 178924 has partial resistance to lettuce drop. Of the two crosses, progeny from Two Star × Eruption have demonstrated better resistance, while the horticultural characteristics are better in PI 178924 × Red Fox. This material has been intercrossed [(Two Star × Eruption) × (PI 178924 × Red Fox)], and F_2 seed was developed for further breeding.

c. Verticillium wilt resistance breeding (with G. Sandoya and K. Subbarao)

Verticillium wilt is a highly destructive soil borne disease caused by the fungus *Verticillium dahliae*. Two races (race 1 and race 2) of the pathogen are known. Resistance to race 1 is provided by the *Vr1* gene. We evaluated a population of eight inbred iceberg breeding lines (RH12-3194 through RH12-3201) from Tiber × ((Pacific × La Brillante) × Tiber) in race 1 *V. dahliae*-infested and non-infested field experiments. The breeding lines carry the *Vr1* gene, and possess a level of resistance to race 1 isolates equivalent to La Brillante. In six Salinas Valley field experiments conducted between 2012 and 2014 and with harvest dates between May and September, the horticultural characteristics and the range of adaptation were most similar to Tiber. Core height, head size, head weight, and shelf-life of salad or whole heads were generally not significantly different from Tiber or Salinas. Yields of the breeding lines and check cultivars varied widely between experiments ranging from 117 cartons per acre (Salinas in a Verticillium wilt infested site) to 1128 cartons per acre (RH12-3199 in a non-infested site). Expressed as a percentage, average yields of breeding lines from all non-infested field experiments ranged from 53% (RH12-3200) to 98% (RH12-3195) of Tiber, while yields from all *V. dahliae* infested field sites ranged from 80% (RH12-3199) to 156% (RH12-3196) of Tiber. The heads of the breeding lines are flatter than Salinas and Tiber. Two breeding lines (RH12-3195 and RH12-3196) had a significantly greater incidence of tipburn than Tiber. Greenhouse and field experiments were conducted to assess resistance to other diseases. The breeding lines are susceptible to bacterial leaf spot, have a similar level of susceptibility to big-vein as Tiber, and appear to be more susceptible to lettuce drop than Tiber and Salinas. A release statement has been written and full public release is pending approval by the USDA-ARS.

We made progress towards developing iceberg breeding lines that combine resistance to race 1 isolates of *V. dahliae* with resistance to other diseases. We evaluated 45 F_3 families in a replicated field experiment for resistance to race 1 *V. dahliae* and corky root. The families are derived from crosses between breeding lines carrying *Vr1* to the corky root resistant cultivars Telluride and Durango. Three families were selected for combining resistance to Verticillium wilt resistance and corky root.

We are working to develop a race 2 infested field site at the USDA research station for germplasm screening, breeding, and genetics research. We conducted another transplanting with susceptible lettuce seedlings inoculated with race 2 isolate VdLs17. We then direct seeded La Brillante and a susceptible iceberg cultivar into the field site to determine if microesclerotia levels are high enough to cause the disease. At harvest maturity, we will collect plant samples to determine the race of the pathogen by using specific primers amplifying race 2 isolates and also collect soil samples for microesclerotia counts.

d. Fusarium wilt

Three races of Fusarium wilt are known in Japan; to date only race 1 is known in California and Arizona. There is now concern of Fusarium wilt and Verticillium wilt pathogens co-infesting fields in south Monterey County. Two field tests of F_4 families from a cross of resistant 'King Louie' × susceptible 'Autumn Gold' were planted in Huron and Yuma. There was essentially no disease expressed in Huron in contrast to Yuma. The mean rating of 'Costa Rica No. 4' and 12 F_4 families at Yuma was 1.0. Mean disease ratings for 'King Louie', 'Salinas' and 'Autumn Gold' were 1.2, 1.9 and 4.0, respectively, at Yuma. Mean disease ratings of 'Cost Rica No. 4' and 12 F_4 families were 1.0 at Yuma. Mean ratings for 'King Louie', 'Salinas', 'Autumn Gold', and 'Patriot' were 1.2, 1.9, 3.7, and 4.0, respectively at Yuma. Approximately 40 Fusarium wilt-resistant F_4 plants that exhibited iceberg type characteristics were dug for seed production in a greenhouse at Salinas, but none survived to produce seed.

The best F_4 family, based on Fusarium resistance and plant type was advanced to the F_5 and backcrossed to 'Light House' to combine high level resistance with a high quality iceberg type adapted to the early fall planting slot in the lower desert. This family was also crossed with the advanced Verticillium wilt-resistant selection RH12-3196 as the initial step to combine resistances to the two pathogens.

e. Lettuce dieback

The lettuce dieback disease is caused by two closely related soilborne viruses of the family *Tombusviridae* – tomato bushy stunt virus (TBSV) and lettuce necrotic stunt virus (LNSV). Previous studies have provided no evidence that either chemical treatment or rotation with non-host crops can effectively reduce, remove, or destroy the virus in infested soil; thus resistant cultivars are the only known protection against the disease. While modern iceberg cultivars are resistant to dieback, susceptibility is widespread in romaine and leaf-type lettuce.

We continued developing romaine and leaf-lettuce breeding lines with combined resistance to dieback and other desirable traits. Crosses have been made to develop material with combined resistance to dieback and downy mildew, tipburn, Verticillium wilt, and extended

shelf-life. Material were grown and tested in field conditions for resistance to downy mildew, and tipburn. The best lines were screened in multiple trials for disease resistance and post-harvest deterioration after minimally processing for salad. Resistance to dieback will be tested with the *Cntg10192* molecular marker that is closely linked to the *Tvr1* gene (Simko et al. 2009, BMC Plant Biology 9:135). Inbred lines with resistance to dieback were released in 2014 (Simko et al., HortScience 49:2014).

f. Bacterial leaf spot (with C. Bull)

The foliar disease bacterial leaf spot is caused by *Xanthomonas campestris* pv. *vitians* (*Xcv*). We have identified a new gene, *Xar1*, that confers resistance to California strains of the bacteria (Hayes et al. 2014, Horticulture Research 1, 14066; doi:10.1038/hortres.2014.66). *Xar1* can be detected by infiltrating the backside of a leaf with *Xcv*. Lettuce with *Xar1* rapidly develops a dry tan spot at the infiltration site, rather than the black watery lesions typical of bacterial leaf spot. The breeding line RH08-0111 was released in 2013 for combining *Xar1* with resistance to race 1 of Verticillium wilt (due to *Vr1*) and resistance to downy mildew. RH08-0111 was crossed to the cultivar Telluride, which is corky resistant, and F_2 progeny were developed from this cross.

We are breeding BLS resistance into cultivars suitable for spring mix production. Previous research determined that the greatest need for BLS resistance is in red romaine and red leaf types. We are developing populations that are genetically uniform for bacterial leaf spot resistance, but variable for leaf color and morphology. This will enable private seed companies to select inbred lines with a range of leaf types, without the need to select for disease resistance. Approximately 450 F_2 progeny from RH08-0111 × Merlot were selected for *Xar1* and red leaf color. Twenty plants were selected and allowed to self-pollinate to produce F_3 families. Further evaluation of F_3 families is needed to identify families that are uniform for *Xar1*, have at least a few plants with dark red color, and possess good shelf life in modified atmosphere packaging.

g. Corky root

We have previously screened more than 1,000 PI lines and cultivars for new sources of resistance to corky root, and four *L. serriola* lines (PI 273597c, PI 491096, PI 491110, and PI 491239) were found highly resistant to the disease. PI 491239 and PI 273597c had lower corky root severity than cultivars with *cor* resistant gene in soil from Watsonville that has *cor*-resistance breaking strains. The resistance from these lines is being incorporated into cultivated lettuces.

We continued to make crosses to transfer resistant gene *cor* from ‘Glacier’ to green leaf, red leaf, romaine, and butterhead lettuce types, and to combine corky root resistance with resistances to other diseases and insects. F_2 to F_6 plants from these crosses were selected in the field for horticultural traits and resistances to corky root, downy mildew, leafminers, and tipburn. Backcrosses were used as necessary to restore horticultural types.

Nineteen F_7 to F_{11} breeding lines of butterhead, red leaf, and red romaine lettuce were tested in a replicated field trial at the USDA Spence Farm in Salinas from June to August 2014 for corky root resistance and horticultural traits. The corky root resistance of the breeding lines

was similar to the resistant controls, while their plant weight, plant height, core length, and tipburn were comparable or better than control cultivars (Tables 1-3).

Table 1. Mean values of corky root severity and head characteristics of butterhead lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2014.

Genotype	Corky root[z]	Plant Wt. (g)	Core length (cm)	Tipburn leaves[y]
Dark Green Boston	7.9 A	325.7 GH	6.7 BCDEF	2.5 A
Margarita	7.6 AB	266.8 H	3.6 G	0.3 B
Cobham Green	7.6 AB	364.4 FG	6.0 DEF	3.6 A
Bibb	7.5 B	365.3 FG	6.5 CDEF	0.7 B
MU13-471	6.1 C	416.3 DEF	8.8 A	0.0 B
MU13-465	5.9 C	409.9 EF	8.4 AB	0.0 B
MU13-466	5.9 C	404.7 EF	7.3 ABCDE	0.0 B
Glacier	5.9 CD	763.7 A	7.8 ABC	0.0 B
MU13-469	5.8 CDE	527.7 BC	8.1 ABC	0.9 B
MU11-534	5.5 DEF	477.3 CDE	5.1 FG	0.0 B
MU10-482	5.4 EF	492.3 CD	5.6 EF	1.1 B
MU10-479-1	5.4 F	576.2 B	7.8 ABCD	1.1 B
MU10-480-1	5.3 F	442.4 DE	6.6 BCDEF	0.2 B
MU10-481-1	5.3 F	432.9 DEF	5.9 EF	0.1 B

[z] Brown and Michelmore's scale of 0-9 (0, no disease; 9, plant is dead from the disease). Means in the same column followed by different letters indicate significant differences at $P < 0.05$.
[y] Number of leaves with tipburn in a head.

Table 2. Mean values of corky root and head characteristics of red leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2014.

Genotype	Corky root[z]	Plant Wt. (g)	Core length (cm)	Tipburn leaves[y]
Red Fox	7.9 A	320.2 C	6.1 ABC	0.0 A
Lolla Rossa	7.7 AB	163.7 EF	7.3 AB	0.0 A
Redina	7.5 BC	131.5 F	5.6 BCD	0.0 A
Prizehead	7.4 BC	225.5 DE	3.7 DE	0.2 A
Merlot	7.2 C	158.2 EF	5.9 ABC	0.0 A
Big Red	7.2 C	233.0 D	4.8 CD	0.0 A
MU12-1032-1	5.9 D	323.7 C	4.9 CD	0.2 A
MU13-480-1	5.9 D	415.8 B	7.3 AB	0.0 A
Glacier	5.9 D	763.7 A	7.8 A	0.0 A
MU12-948-1	5.9 D	405.0 B	7.0 AB	0.0 A
MU13-414-1	5.8 D	143.8 F	2.2 E	0.0 A
MU11-332	5.7 D	195.7 DEF	2.7 E	0.0 A

[z]Brown and Michelmore's scale of 0-9 (0, no disease; 9, plant is dead from the disease). Means in the same column followed by different letters indicate significant differences at $P < 0.05$.
[y] Number of leaves with tipburn in a head.

Table 3. Mean values of corky root severity and head characteristics of red romaine lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2014.

Genotype	Corky root[z]	Plant height (cm)	Plant Wt. (g)	Core length (cm)	Tipburn leaves[y]
Green Forest	8.0 A	28.5 B	787.4 A	9.0 BC	0.0 B
Red Hot Cos	7.9 A	26.8 BC	447.2 D	5.7 C	0.0 B
Parris Island	7.8 A	25.4 C	475.7 CD	7.4 BC	0.0 B
Flashy Troutback	7.7 A	26.8 BC	628.5 ABC	20.3 A	0.4 A
Heart's Delight	6.4 B	26.7 BC	459.2 D	8.5 BC	0.0 B
Clemente	5.9 BC	26.8 BC	524.5 BCD	7.6 BC	0.0 B
MU11-470-2	5.7 CD	32.1 A	660.0 AB	9.0 BC	0.0 B
MU13-405	5.6 CD	26.1 BC	479.0 CD	10.7 B	0.1 B
MU11-449-1	5.6 CD	26.4 BC	515.8 BCD	7.9 BC	0.0 B
MU13-404	5.6 CD	25.2 C	365.5 D	8.3 BC	0.0 B
MU12-882	5.4 D	24.0 C	381.4 D	5.8 C	0.0 B

[z] Brown and Michelmore's scale of 0-9 (0, no disease; 9, plant is dead from the disease). Means in the same column followed by different letters indicate significant differences at $P < 0.05$.
[y] Number of leaves with tipburn in a head.

h. Downy mildew (with R. Michelmore, M. Truco, O. Ochoa, R. Antonise, M. Pel)

Downy mildew (caused by the oomycete *Bremia lactucae*) is considered by some to be the most important disease affecting lettuce production world wide. Over 40 resistance genes (*Dm* genes) have been identified and introgressed into cultivated lettuce. Although *Dm* genes can be used in the resistance breeding programs they are race-specific and thus can be defeated by new isolates of the pathogen. Our research focuses on developing material with quantitative resistance. Material with this type of resistance (often called field resistance) is usually infected with the pathogen, but there are fewer and smaller lesions on fewer affected leaves, and disease progresses at a slower rate than on susceptible cultivars.

Five mapping populations have been being developed and are currently being tested in replicated field trials to detect quantitative trait loci for downy mildew resistance. Those populations originated from the crosses Salinas (susceptible) × Grand Rapids (resistant), PI 491224 (susceptible) × Iceberg (resistant), Grand Rapids × Iceberg, Salinas 88 (susceptible) × La Brillante (resistant), and Parade (intermediate) × Pavane (susceptible). Two populations based on crosses between Grand Rapids × Iceberg, Salinas 88 × La Brillante were previously genotyped with SNP (R. Michelmore's laboratory) and AFLP (KeyGene, The Netherlands) markers, while the Parade × Pavane population was genotyped with SNP markers. Field-based testing confirmed the presence of polygenes for resistance to downy mildew in all populations. Some lines in the Grand Rapids × Iceberg population exhibited either higher or lower levels of resistance than the two parents.

We previously determined that Batavia type cultivar La Brillante has a high level of field resistance to the disease in California. The Salinas 88 × La Brillante mapping population has been tested in five field trials and four laboratory experiments. The presence of a new, dominant

resistance gene (designated *Dm50*) that confers complete resistance to specific isolates of *Bremia lactucae* in cv. La Brillante was detected in laboratory tests of seedlings inoculated with multiple diverse isolates. *Dm50* is located in the major resistance cluster on linkage group 2 that contains at least eight major, dominant *Dm* genes conferring resistance to downy mildew. This *Dm* gene is, however, ineffective against the isolates of *B. lactucae* prevalent in the field in California and the Netherlands. A quantitative trait locus (QTL) located at the *Dm50* chromosomal region (*qDM2.2*) was detected, though, when the amount of disease was evaluated a month before plants reached harvest maturity. Four additional QTLs for resistance to *B. lactucae* were identified on linkage groups 4 (*qDM4.1* and *qDM4.2*), 7 (*qDM7.1*), and 9 (*qDM9.2*). The largest effect was associated with *qDM7.1* (up to 32.9% of the total phenotypic variance) that determined resistance in multiple field experiments. Markers identified in the present study will facilitate introduction of these resistance loci into commercial cultivars of lettuce. (Fig. 2).

The Grand Rapids × Iceberg population has been tested in five field trials and two laboratory experiments. Field experiments were conducted from 2008 to 2013 in Salinas and The Netherlands. Three significant QTLs were detected in all environments. The QTLs located on linkage groups 2 (*qDM2.1*) and 5 (*qDM5.1*) were associated with disease resistance under both field and laboratory conditions. Resistance alleles at both QTLs originated from the cultivar Iceberg. The QTL on LG 9 (*qDM9.1*) was detected through simultaneous analysis of all experiments using a mixed-model approach. Alleles for elevated resistance at this locus originated from the cultivar Grand Rapids. Markers linked to the resistance alleles can aid future marker-assisted selection programs, thus complementing field-based phenotyping. We are developing additional mapping populations from crosses with highly susceptible accessions that will be used to map resistance genes not detected in the present studies.

Crosses were made to develop new breeding lines that would exploit field resistance observed in the cultivars Balady Banha, Iceberg, Grand Rapids, Holborn's Standard, La Brillante, Merlot, and Primus. The hybrid plants were detected with molecular markers developed by our laboratory. Selections from multiple families will be evaluated in replicated trials for resistance to downy mildew, bolting, tipburn, and horticultural characteristics. These selections were made from spring and summer plantings in Salinas. Plants were selected that had a minimum number of lesions and were not bolting at the time of evaluation. The selected material will be evaluated for yield, size, uniformity, and tipburn resistance. A good level of resistance to downy mildew was observed in material originating from crosses with Balady Banha, Iceberg, and Grand Rapids.

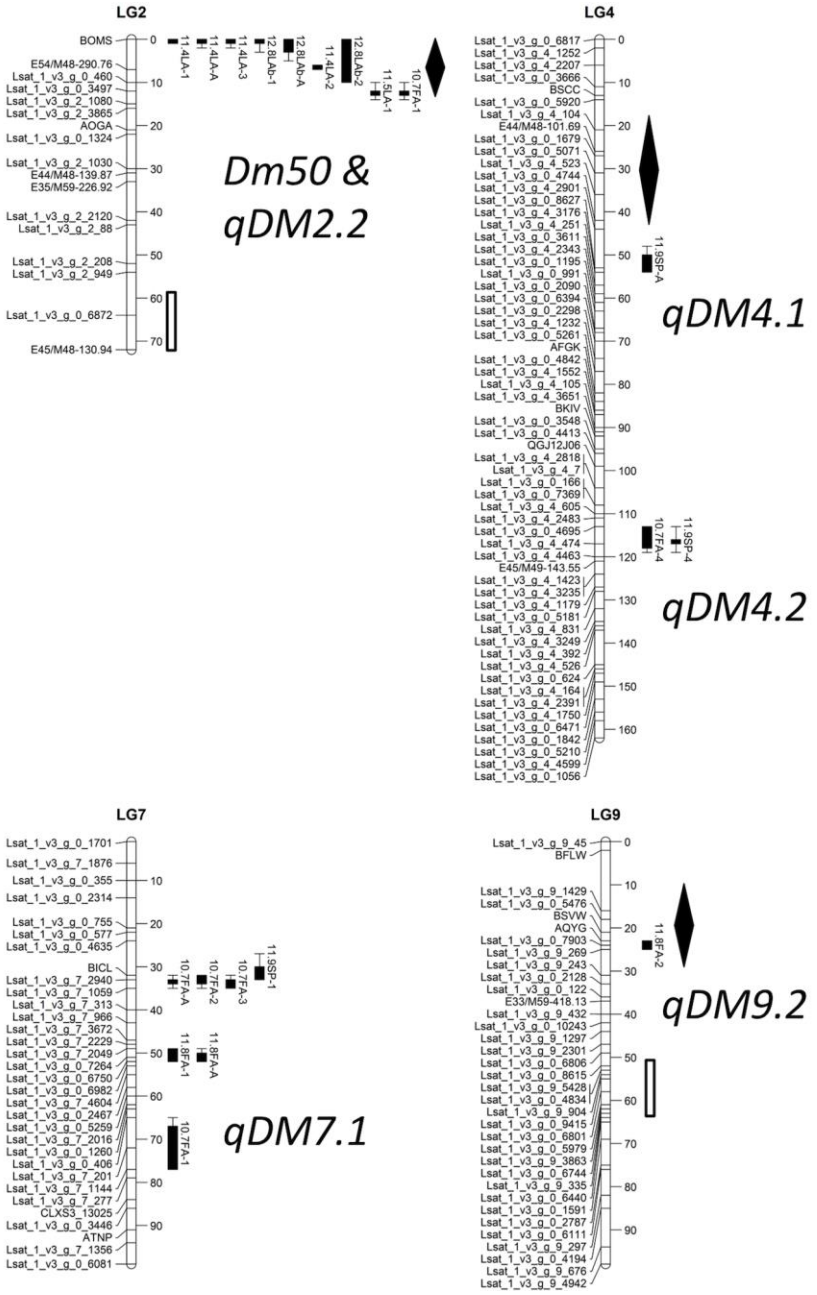

Fig. 2. Locations of five loci for resistance to downy mildew on the 'Salinas 88' × 'La Brillante' genetic linkage map. Black bars indicate 1-LOD support interval around significant loci on LG2 (*Dm50/qDM2.2*), LG4 (*qDM4.1* and *qDM4.2*), LG7 (*qDM7.1*) and LG9 (*qDM9.2*). Whiskers on bars correspond to 2-LOD support intervals. Black diamonds indicate approximate positions of the major resistance gene clusters. The cluster on LG2 harbors *Dm1*, *Dm2*, *Dm3*, *Dm6*, *Dm14*, *Dm15*, *Dm16*, and *Dm18* conferring resistance to downy mildew, *Xar1* gene for resistance to bacterial leaf spot, and *Ra* gene for resistance to lettuce root aphid. The cluster on LG4 harbors *Dm4*, *Dm7*, *Dm11*, *Dm44*, *Dm48*, and *Dm49* for lettuce resistance to downy mildew. The cluster on LG9 harbors *Dm39* for resistance to downy mildew and *Vr1* for resistance to Verticillium wilt. White bars show positions of QTLs for resistance to downy mildew detected in a 'Grand Rapids' × 'Iceberg' (GR × Ice) mapping population; *qDM2.1* on LG2 and *qDM9.1* on LG9. Scale is in Kosambi centimorgans (cM). Figure is from Simko et al., Phytopathology, 2015.

2. Insect resistance

a. Leafminer

Crosses were made to transfer leafminer resistance from wild species into iceberg and mixed lettuce types. BC_1F_2 to BC_1F_6 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. Crosses were also made among resistant sources to elevate the level of resistance.

F_2 to F_6 plants from crosses between leafminer resistant 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. Ten promising F_7 to F_{11} breeding lines of green leaf, red leaf, and romaine lettuce were trialed at Spence Farm in Salinas from June to August 2014 with four replications, along with check cultivars. The breeding lines all had significantly lower leafminer sting density than cultivars and resistant controls, and the plant weight, plant height, core length, and tipburn of many lines were similar to or better than commercial cultivars (Tables 4-6). Some of these lines also showed resistance to corky root. These breeding lines will be evaluated again next year.

Table 4. 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 2014.

Genotype[z]	Stings/cm^2[2,y]	Plant Wt. (g)	Core length (cm)	Corky root[x]
Two Star	7.1 A	297.0 ABC	4.8 B	7.9 A
Waldman's Green	6.5 A	325.7 AB	6.8 A	8.0 A
Grand Rapids	4.2 B	235.0 C	4.5 B	8.0 A
Shining Star	3.6 B	247.7 BC	4.9 B	8.0 A
MU11-365-1 (*cor*)	1.4 C	290.3 ABC	4.2 B	5.9 B
MU13-339 (*cor*)	1.2 C	335.3 A	4.1 B	5.9 B
MU11-373 (*cor*)	1.1 C	266.3 ABC	3.6 B	5.8 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] On Brown and Michelmore's scale of 0-9 (0, no disease; 9, plant is dead from the disease).

Table 5. 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 2014.

Genotype	Stings/cm^{2,z}	Plant Wt. (g)	Core length (cm)	Tipburn leaves^{y}
Red Fox	7.1 A	320.2 A	6.1 AB	0.0 A
Big Red	6.9 A	233.0 BC	4.8 BC	0.0 A
Prizehead	5.6 B	225.5 BC	3.7 C	0.2 A
Lolla Rossa	3.6 C	163.7 C	7.3 A	0.0 A
Merlot	3.4 C	158.2 C	5.9 ABC	0.0 A
MU07-838	1.6 D	239.5 ABC	4.8 BC	0.0 A
MU11-368-1	1.5 D	293.3 AB	6.2 AB	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 6. 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 2014.

Genotype^{z}	Stings/cm^{2,y}	Plant Wt. (g)	Core length (cm)	Height cm
Green Forest	12.8 A	787.4 AB	9.0 BC	28.5 AB
Parris Island	11.1 A	475.7 C	7.4 C	25.4 B
Heart's Delight	8.8 B	459.2 C	8.5 BC	26.7 AB
Red Hot	5.3 C	447.2 C	5.7 C	26.8 AB
MU12-1223-1 (*cor*)	2.5 D	601.3 BC	10.5 BC	27.0 AB
MU11-572-1 (*cor*)	2.4 D	551.5 BC	8.9 BC	28.2 AB
MU11-570-1	2.4 D	798.5 AB	12.4 AB	29.0 A
MU13-386-1 (*cor*)	1.9 D	997.0 A	15.5 A	29.7 A
MU11-506	1.2 D	596.5 BC	6.9 C	27.3 AB

^{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 $P < 0.05$.

b. Lettuce aphid

Two biotypes are known: Nr:0, which is known world wide, and Nr:1, which is known only in Europe. Resistance to biotype 0 (Nr:0) is controlled by a multiple allelic series of genes at a single locus. Complete (high-level) resistance in *L. virosa* PIVT 280 is controlled by the single dominant gene Nr^{C} that is being transferred to U.S. types by commercial breeders. Partial resistance in *L. serriola* PI 491093 is controlled by the single dominant allele Nr^{P} that is recessive to Nr^{C} but dominant to susceptibility, which is conditioned by the recessive allele *nr*. Partial resistance was expressed in open field tests, and in controlled-infestation field studies. Additional germplasm was obtained for testing: 55 CGN lettuce accessions reported to express

resistance against European strains of biotypes Nr:0 and Nr:1. Seed of most of the accessions was increased in a greenhouse.

Aphid resistant selections were crossed with 'Salinas', 'Vanguard 75', 'Parris Island Cos', and 'Waldman's Green' to produce the second backcross (BC_2) generation to 'Salinas', and the first backcross to 'Vanguard 75', 'Parris Island Cos', and 'Waldman's Green' following the first backcross to 'Salinas'. Unusually hot temperatures in 2014 resulted in loss of aphid colonies. They were re-established through the winter for testing in 2015.

3. Adaptation and Quality

a. Bolting resistance for fall plantings (with D. Still)

Research on bolting resistance for fall plantings is being conducted collaboratively with Dr. David Still from California State Polytechnic University, Pomona. Funding for the collaborative research comes from the Agricultural Research Initiative with matching funds from the California Leafy Greens Research Program. A mid-September planted field experiment was conducted to evaluate 12 romaine advanced inbred lines and six romaine cultivars for resistance to pre-mature bolting and horticultural quality. Premature bolting was observed in at least a few plants of all lines, while it was ubiquitous and severe in some cultivars. Siskiyou and three breeding lines had an average core length under 3.5 inches. Average core length for the remaining entries ranged from 4 inches to over 20 inches. The best performing breeding lines were derived from crosses involving Siskiyou and Blonde Lente a Monter.

Recombinant inbred line populations from Salinas 88 × PI 251246, Pavane × Parade, Western Red Leaf × Cool Guard, and Diplomat × Margarita were evaluated for plant development on a scale of 1 = rosette; 3 = bolting; 5 = flowering; 7 = open involucres with seed in a mid-September planted experiment in Yuma, AZ and a July planted field experiment in Salinas, CA. In total, these populations have been evaluated for plant development in two experiments located in Yuma, AZ and in three California experiments. We will collect development data from one additional field experiment. We have also collected development data from growth chamber experiments using Salinas 88 × PI 251246 RILs grown under four day-length x temperature combinations. The data will be used to map QTL for plant development in the lettuce genome and determine which QTL are environmentally sensitive.

b. Tipburn

We are developing improved tipburn resistance in romaine cultivars adapted to coastal and desert production using the tipburn resistance found in modern iceberg cultivars. We developed inbred romaine breeding lines from Green Towers × Salinas with consistently less tipburn than Green Towers in four coastal and four low desert replicated field experiments. Core height and head weight in this material is generally similar to Green Towers, though the overall horticultural type of this material is less desirable than Green Towers. Seed of nine F_6 and two F_7 lines are being increased for further testing. Thirty-seven F_5 lines from Clemente × Hallmark were evaluated for tipburn resistance, head weight, and core height in a single replicated low desert experiment. None of the lines had adequate performance and all were discarded. New F_1 hybrids were

produced from Beacon × King Henry, Gabilan × King Henry, Gabilan × Green Towers, Lighthouse × King Henry, and Lighthouse × Green Towers.

We have identified a virescent mutant of PI 129535 (PI 129535vir), a character where leaves remain blanched (light green or yellow) for an extended period of development despite being exposed to light. Older leaves of PI 129535vir eventually turn a normal green color, but much later in development than wild-type plants. Previous research has shown that romaine cultivars that remain open at the top of the plant develop less tipburn than hearting type romaine (Jenni and Hayes, 2010, Euphytica 171:427–439). However, open top romaine plants may be less desirable since they do not produce blanched inner leaves. Therefore virescence may allow decreases in tipburn by enabling the development of cultivars with open tops that still possess blanched inner leaves. The genetics of virescence was previously described as a single recessive gene named *virescence* (*vir*), originating in USDA breeding line M400-27 (Ryder, 1971, Journal of the American Society for Horticultural Science 96:826-828). In 2014, were determined that virescence in PI 129535vir segregated as a single recessive gene in F_2 and BCF_2 populations. We are producing BCF_3 seed from over 200 virescent selections for use in 2016 field experiments.

B. Genetic studies

1. Shelf life of fresh-cut lettuce (with C. Galeano, C. F. Forcada and R. Michelmore)

We previously identified a major QTL for decay of fresh-cut lettuce (*qSL4*) on linkage group 4 in the Salinas 88 × La Brillante recombinant inbred line population (Hayes et al. 2014. Journal of the American Society for Horticultural Science 139:388-398). The La Brillante allele of this QTL results in rapid decay of fresh-cut lettuce. Dr. C. Galeano and later Dr. Carolina Font Forcada working at UC-Davis as part of the Specialty Crop Research Initiative Project 'Genes to Growers' have been working to fine map and clone the gene controlling this QTL. Cloning the gene will increase our understanding of the biology of the trait and aid in the development of markers useful for marker assisted selection. To accomplish this goal, UC-Davis developed a population of F_3 families to fine map *qSL4* within a genomic region of three molecular markers known to be closely linked to the QTL. The USDA grew 48 of these F_3 families and known shelf-life controls in a Yuma, AZ field experiment. The heads were harvested, vacuumed cooled, and shipped to Salinas, CA on January 15 and 16. Twenty plants per family and control lines were processed into salad on January 18 and evaluated for shelf-life once per week for the next seven weeks using a 0 (no decay) to 10 (100% decay) scale. Each plant was used to make a single bag of salad. The shelf-life data was provided to UC-Davis for analysis.

2. Verticillium wilt (with G. Sandoya and R. Michelmore)

Race 1: We previously determined that resistance to race 1 isolates in La Brillante is due to the single dominant gene *Verticillium resistance 1* (*Vr1*) found on linkage group 9. However, multiple sources of race 1 resistance are known. Allelism experiments using F_2 plants from crosses of La Brillante × Annapolis (283 progeny), La Brillante × Eruption (288 progeny), La Brillante × Little Gem (291 progeny), La Brillante × Merlot (284 progeny), and La Brillante ×

Pavane (297 progeny) tentatively indicate that all these cultivars carry *Vr1* or a gene closely linked to *Vr1*. More testing is needed to confirm this finding.

Vr1 maps to a region of the lettuce genome containing gene sequences similar to the *Ve* gene in tomato. *Ve* confers resistance to race 1 isolates of *V. dahliae* in tomato by interacting with the *Ave1* protein expressed by race 1 isolates. *V. dahliae* isolates lacking *Ave1* are able to cause disease despite the presence of *Ve*, and are therefore race 2 isolates. It is hypothesized that the lettuce *Vr1* gene operates through a similar mechanism. We conducted screening of race 2 *V. dahliae* isolates that were transformed to express the *Ave1* gene. VdLs16 and VdLs17 wild type isolates of races 1 and 2 respectively were used as controls. The transformed isolates caused very little disease. Analysis using RQ-PCR with transformed isolates showed little or no expression of *Ave1*, therefore the use of these transformed isolates was not pursued further.

Race 2. Ninety-nine F_3 families from the cross Salinas × PI 171674 were evaluated in a single greenhouse experiment with 5 replications arranged in an alpha-lattice design. Parents, PI 171674 and Salinas were also included in the experiment and replicated 18 times. Disease severity (DS, 0=no root discoloration to 5=complete root discoloration), the percentage of the plant with foliar symptoms (FS), and days to first flower (DFF) were assessed on each plant. The frequency of plants with DS $\geq$ 1 was used to calculate the disease incidence (DI). Family means ranged from 8% to 71% for DI and 0.4 to 3.4 for DS. Parents significantly differed from each other having means of 15% and 55% for DI and 0.7 and 2.7 for DS for PI 171674 and Salinas, respectively. The presence of segregation and high-level resistance in this cross makes this population useful for identifying quantitative trait loci for resistance. Two families showed significantly lower disease compared to Salinas. These families could be used to initiate breeding of iceberg cultivars with race 2 resistance.

We are collaborating with UC Davis to evaluate a recombinant inbred line (RIL) population from the cross PI 251246 (*L. sativa* oil-seed lettuce) × 11G99 (Armenian *L. serriola* with partial resistance to race 2). This greenhouse experiment will provide a second environment of disease resistance data for this population. The data will be used for mapping resistance QTL.

We are intercrossing resistant accessions to develop populations that could have higher levels of resistance than is currently known. We crossed the *L. serriola* accession 11G99 with three *L. sativa* PIs (169511, 171674, and 204707). PI 171674 and 11G99 appear to have the highest resistance among these accessions and we are currently developing a population of F_3 families from 11G99 × PI 171674.

C. Molecular markers

1. Shelf-life (with R. Michelmore and M. Truco)

We continue developing molecular markers that can be used for marker-assisted selection of germplasm with good shelf-life. Analysis of the sequenced lettuce genome yielded 35 scaffolds with high similarity to the AFLP marker that is closely linked to *qSL4*. We have developed primers that amplify 80 genomic regions from these 35 scaffolds. Amplification was first conducted on four cultivars with very good or very poor shelf-life of salad-processed lettuce (two cultivars in each group). Sequencing revealed single nucleotide polymorphisms (SNPs) among the four cultivars. Twenty amplicons that showed perfect matches between SNPs and phenotypic data were used in the subsequent analyses. These 20 genomic regions were sequenced across

eighteen cultivars with different quality of shelf-life. We developed assays for the high-resolution DNA melting (HRM) analysis and Kompetitive Allele Specific PCR (KASP) genotyping. The marker-trait analysis was subsequently performed on 153 accessions from different horticultural types of lettuce. Neither one of the markers showed a perfect match with shelf-life phenotypes, however a combination of two markers flanking *qSL4* was able to distinguish accessions with very fast tissue decay after processing for salad. In 2014 we have grown in the field twelve sets of F_2 families with 50 plants per family. These families are originating from crosses between parents with very fast decay and slow or intermediate speed of decay. All families and their parents were processed for salad, and evaluated for decay by the method developed at our research station (Hayes et al., Journal of the American Society for Horticultural Science, 2015). After processing, decay data was collected every 7 days for 63 days using a 0 (no decay) to 10 (100% decay) scale. All families exhibited genetic variation (segregation) for decay. Genotyping of this material will allow us to determine the accuracy of the markers and their potential for marker-assisted selection.

Screening cultivars with markers linked to *qSL4* suggests that *qSL4* conditions fast decay in several other fast decaying cultivars and accessions (Little Gem, Triple Threat, PI 491224, Big Boston, Bibb, Gallega, and another 20 accessions). These cultivars or accessions possess the molecular markers linked to the rapid decay QTL present in La Brillante. Still, several other fast decaying accessions possess marker profiles indicative of slow decay. These included Balady Barrage, Big Red, Red Eye Cos, PI 491209, Shinning Star, Solito, and *L. serriola* UC96US23. This outcome may be due to weak linkage between the molecular markers and the gene for shelf-life, or due to a unique genetic basis for rapid decay in these accessions. We crossed these accessions to La Brillante to develop populations for allellism experiments. F_2 populations for use in field experiments will be available in late 2015. The allelism experiments will tell us whether these cultivars have the same gene for rapid decay as La Brillante or have a different gene.

D. Automatic detection of decay in fresh-cut lettuce (with JA Jimenez-Berni and RT Furbank)

Fresh-cut lettuce sold in modified atmosphere packaging (MAP) is a desirable, but highly perishable product. Decay of tissue can start a few days after processing and may be difficult to detect by quick visual observation. A system for early detection of decay and gradual evaluation of its progress is important both for lettuce processing industry and for breeding companies and institutions assessing quality of new cultivars and breeding lines. We have developed two lettuce decay indices (LEDI) that can be used to detect decay of leaf tissue. One of the indices ($LEDI_4$) is based on three wavelengths identified from hyperspectral imaging, while the second index ($LEDI_{CF}$) is based on chlorophyll fluorescence imaging (Fig. 3). In addition to detecting lettuce decay, the indices identified tissue damaged by freezing temperatures. $LEDI_4$ and $LEDI_{CF}$ showed almost 97% accuracy in classifying tissue as being fresh or decayed when tested on red, dark green, green, light green, and yellow leaves. Specificity of the indices decreased when tested on fresh tissue with a very limited amount of chlorophyll that visually appeared to be almost white. Both indices detected lettuce decay without opening plastic MAP bags. The non-destructive nature of the methods thus allows rapid and repeated evaluation of samples over time and presents the opportunity for development of a commercial, high throughput scanner for evaluation of bagged, fresh-cut lettuce quality.

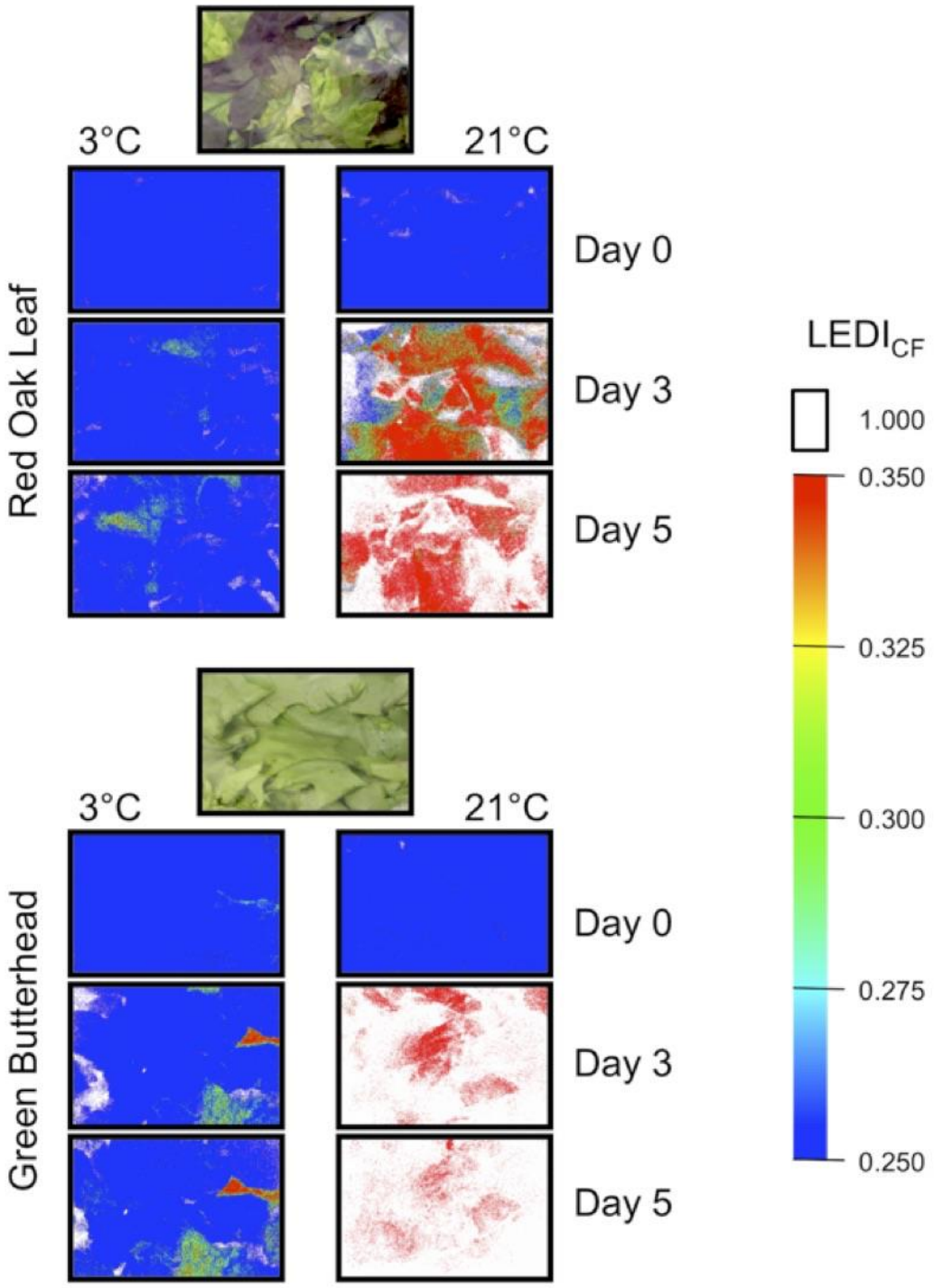

Fig. 3. Analysis of lettuce decay in plastic bags used for modified atmosphere packaging. Two types of lettuce, red oak leaf and green butterhead from the evaluation set 3, were fresh-cut and stored in dark at 3 °C or 21 °C. Decay of tissue was analyzed on day 0 (processing), 3, and 5. Shown are the results from the chlorophyll fluorescence imaging that were used to calculate $LEDI_{CF}$. Dark blue color indicates fresh tissue, while tissue considered to be decayed is in white color. Light blue, green, yellow, orange, and red colors implies intermediate category with the gradually increasing probability that the tissue is decayed. Figure is from Simko et al., Postharvest Biology and Technology, 2015.

E. Survival of human enteric pathogens on lettuce (with M Brandl)

Downy mildew, a plant disease caused by the oomycete *Bremia lactucae*, is endemic in many lettuce-growing regions of the world. Invasion by plant pathogens may create new portals and opportunities for microbial colonization of plants. The occurrence of outbreaks of *Escherichia coli* O157:H7 (EcO157) and *Salmonella enterica* infections linked to lettuce prompted us to investigate the role of downy mildew in the colonization of romaine lettuce by these human pathogens under controlled laboratory conditions. Whereas both EcO157 and *S. enterica* population sizes increased 10^2-fold on healthy leaf tissue under conditions of warm temperature and free water on the leaves, they increased by 10^5-fold in necrotic lesions caused by *B. lactucae*. Confocal microscopy of GFP-EcO157 in the necrotic tissue confirmed its massive population density and association with the oomycete hyphae (Fig. 4). Multiplication of EcO157 in the diseased tissue was significantly lower in the RH08-0464 line with a high level of resistance to downy mildew than in the more susceptible cultivar Triple Threat. qRT-PCR quantification of expression of the plant basal immunity gene PR-1, revealed that this gene had greater transcriptional activity in line RH08-0464 than in cultivar Triple Threat, indicating that it may be one of the factors involved in the differential growth of the human pathogen in *B. lactucae* lesions between the two accessions. Additionally, downy mildew disease had a significant effect on the colonization of EcO157 at high relative humidity (RH 90-100%) and on its persistence at lower RH (65-75%). The latter conditions, which promoted overall dryness of the lettuce leaf surface, allowed for only 0.0011% and 0.0028% EcO157 cell survival in healthy and chlorotic tissue, respectively, whereas 1.58% of the cells survived in necrotic tissue. Our results indicate that downy mildew significantly alters the behavior of enteric pathogens in the lettuce phyllosphere and that breeding for resistance to *B. lactucae* may lower the increased risk of microbial contamination caused by this plant pathogen.

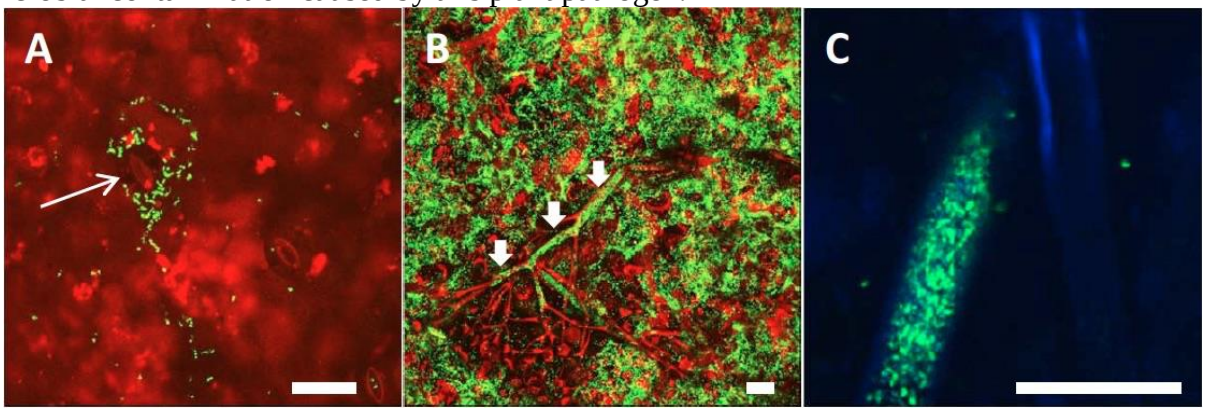

Fig. 4. Confocal micrographs of GFP-EcO157 cells colonizing romaine lettuce leaf tissue (cv. Green Towers). (A) Sparse colonization of healthy tissue by EcO157 with greater proliferation around a stomate (long arrow). (B) High density of EcO157 cells in the necrotized tissue due to infection with the plant pathogen *B. lactucae*, and association of the human pathogen with the oomycete hyphae (short arrows). The large masses of GFP-cells are evidenced by the green fluorescent signal. The yellow signal results from the GFP fluorescence of the bacterial cells co-localizing with the red autofluorescence of the leaf or of the oomycete. (C) Single optical scan longitudinally across a hypha revealing that the GFP-EcO157 cells invaded the oomycete tissue either actively, or passively via damages to the hyphal wall. In contrast, the hypha to the right remained uncolonized. In this image, the signal acquired in the red and far red was pseudo-colored blue. The left and middle images are pseudo-3D projections of multiple optical slices in the *z* range. Bars, 20 µm. Figure is from Simko et al., BMC Microbiology, 2015.

F. Release of breeding lines

We fully characterized a population of eight inbred iceberg breeding lines (RH12-3194 through RH12-3201) from Tiber × ((Pacific × La Brillante) × Tiber) in race 1 *V. dahliae*-infested and non-infested field experiments. The breeding lines carry the *Vr1* gene, and possess a level of resistance to race 1 isolates equivalent to La Brillante. In six Salinas Valley field experiments conducted between 2012 and 2014 and with harvest dates between May and September, the horticultural characteristics, yield, quality, and the range of adaptation were generally similar to Tiber. A release statement has been written and full public release is pending approval by the USDA-ARS.

G. Germplasm evaluation, maintenance and use

1. Screening

a. Bacterial leaf spot (with C. Bull)

In limited screening using *Xanthomonas campestris* pv. *vitians* (*Xcv*) isolates from C. Bull's collection, the *Xar1* gene found in La Brillante was effective against many California isolates but was defeated (resulting in a susceptible reaction) by a single Canadian isolate (BS3127). We screened diverse *Lactuca* accessions in the USDA, Salinas germplasm collection for resistance to BS3127 by infiltrating *Xcv* into leaves of seedlings using a needleless syringe and assessing for a hypersensitive response (HR) 24 to 30 hours of infiltration. This is a pre-emptive effort to identify resistance should BS3127 or a similar isolate be found in California. We detected an HR reaction in ten *L. serriola* accessions. Two greenhouse experiments were conducted with these accessions along with La Brillante, Little Gem, Pavane, Batavia Reine des Glaces, and Vista Verde to assess disease severity against BS3127 and BS347. *Xcv* was sprayed onto four-week-old seedlings and disease severity was evaluated seven days later on a 0 = no disease to 5 = severe disease scale. La Brillante, Little Gem, Pavane, Batavia Reine des Glaces, and Vista Verde were susceptible to BS3127 with mean DS > 2.0. Seven of the *L. serriola* accessions were highly resistant to BS3127 (mean DS < 0.5), while three were highly diseased (mean DS > 2). All ten *L. serriola* accessions were susceptible to BS347 (mean DS > 1.2).

b. Verticillium wilt (with G. Sandoya, K. Subbbarao, and R. Michelmore)

We continued to screen the USDA collection of *Lactuca* germplasm in search of resistance to race 2 isolates of *V. dahliae*. To date, complete resistance to race 2 has not been discovered. One hundred and forty four previously untested cultivars were evaluated for resistance against race 2 isolate VdLs17 in a greenhouse experiment during 2014. PI 171674 and cv. Salinas were included as resistant and susceptible checks. Significant differences were detected among the controls and cultivars. While no cultivar was better than PI 171674, accessions Fle8037, SAL178, Romaine Chicon des Charentes and Petra had a significant lower DI compared to susceptible Salinas. PI 171674 had DI of 39% and Salinas reached 77% DI. We will be retesting these cultivars to confirm resistance.

We conducted two greenhouse experiments testing 98 and 34 cultivars for resistance against VdLs17 in a RCBD design with three replicates. This material had previously shown promise for resistance. Among these accessions, PI 358038 had median DIs of 0% in both experiments with maximum DIs of 20%, while susceptible Salinas had median DIs of 20% and 65% and maximum DIs of 25% and 100%. An additional experiment with eight replications was conducted to test PI 358038, PI 171674, accession 11G99, and Salinas. PI 171674 and 11G99 showed significantly lower DI and DS compared to Salinas. PI 358038 was not different from Salinas however.

We are exploring alternate approaches to decrease disease severity or delay symptom expression past harvest maturity in iceberg cultivars. We rescreened diverse iceberg cultivars to determine if any can delay disease symptoms past market maturity. We evaluated 34 iceberg cultivars from different origins (United States and Europe) along with the race 1-resistant breeding line RH11-1798 and the susceptible Salinas as controls. The experiment was conducted in an artificially infested race 1 field at the USDA-ARS in Salinas, California. We evaluated disease severity (DS; 0 = no root discoloration to 5=complete root discoloration) and the frequency of plants with DS $\geq$ 1 was used to calculate the disease incidence (DI). Foliar symptoms (FS) were evaluated on a scale of 0 = completely healthy to 5 = all cap leaves wilted and/or dead. Head maturity (HM) was rated on a scale of 0 = open heads to 5 = splitting and over mature heads. FS and HM were assessed at three time-points. We confirmed the results from previous experiments that this population has genetic variation for DI, DS, FS and HM. To be more specific, several iceberg cultivars can delay the onset of foliar symptoms. The cultivars that delay symptom expression are not adapted to summer production in the Salinas Valley. We have crossed the cultivars that delay foliar symptoms past market maturity with those that quickly develop wilting to study the genetics of this trait and to determine if delayed symptom expression can be breed into iceberg lettuce adapted to the Salinas Valley.

2. Collection and distribution

We have distributed publicly available accessions, cultivars and populations to various research groups and seed companies worldwide through individual requests and the Organic Seed Partnership program. Released USDA germplasm has been distributed to parties providing written requests. From 2014 through 2015, requests were made for bacterial leaf spot resistant baby leaf populations (RH12-3370 and RH12-3371), Verticillium wilt resistant iceberg breeding lines (RH05-0336, RH05-0339, RH05-0340, RH08-0472, RH08-0475), dieback resistant romaine lines SM09A and SM09B, crisphead lettuce breeding lines with resistance to corky root and lettuce mosaic virus (04-0344, 04-0350, 04-0353, 04-0363, 04-0368, 04-0375, and 04-379), leaf lettuce breeding lines with corky root resistance (06-831 and 06-833), and sixteen inbred lines released in 2014 (SM13-I1, SM13-I2, SM13-I3, SM13-I4, SM13-I5, RH08-0111, SM13-R1, SM13-R2, SM13-R3, RH08-0464, SM13-L1, SM13-L2, SM13-L3, SM13-L4, SM13-L5, and SM13-L6).

We collaborated with the USDA, Plant Exchange Office to collect germplasm in the center of diversity for *Lactuca*. We identified locations in Azerbijian that were likely to have wild *Lactuca*. Exploration and collection of seeds was conducted in 2014 by Prof. Aydin Asgarov (head of Department of Geobotany and Taxonomy, taxonomist, Genetic Resources Institute of ANAS), Niyazi Quliyev (Vegetable researcher of the Department of Vegetable and

Melon Genetic Resources, Genetic Resources Institute of ANAS), and Mahammad Eldarov (collector, scientific worker of the department of molecular cytogenetics). Ninety-seven collections were made and the seeds were sent to the USDA in Pullman, WA to be cataloged as accessions.

H. Evaluation of advanced breeding lines

To fully characterize the lines we develop prior to release, we have developed an extensive adaptation, disease resistance, and quality testing network. These testing resources enable us to characterize and breed multiple resistant cultivars, and to develop a thorough profile of a breeding line's strengths and weaknesses before it is released. Ultimately, this should increase adoption and use of USDA germplasm. Several field trials were planted and evaluated in the Salinas Valley and Yuma. We are indebted to numerous growers and shippers for their cooperation in providing space and resources for our trials. Most evaluation methods we use are documented in previous germplasm release publications: Mou et al. 2007, HortScience 42:701-703; Simko et al. 2010, HortScience 45:670-672; Hayes et al. 2011, HortScience 46:501-504; Simko et al. 2012, Crop Science 52:2131-2142. Arrival quality is being conducted by harvesting, cooling, and shipping lettuce cartons to Dr. Yaguang Luo with the USDA in Beltsville, MD. Whole heads are assessed for quality on a 1 (high quality) through 10 (low quality) scale after 7, 14, and 21 days storage at 5°C. Cooling and shipping is conducted in coordination with Taylor Farms. Data will be accumulated over years and communicated when lines are released. In 2014, we characterized 34 iceberg, 3 leaf, and 3 romaine USDA or UCD breeding lines for yield, horticultural characters, shelf-life of salad and arrival quality, and for resistance to Verticillium wilt, lettuce drop, downy mildew, and tipburn.

I. Recent (2014 - 2015) publications relevant to this project

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