

ABSTRACT
CALIFORNIA LETTUCE RESEARCH BOARD

For the period
(April 1, 2011-March 31, 2012)

PROJECT TITLE: Lettuce Breeding, USDA-ARS

PROJECT INVESTIGATORS: R. Hayes, I. Simko, B. Mou, J. D. McCreight, USDA-ARS Crop Improvement and Protection Unit, Salinas, CA

SUMMARY:

Our objectives are to incorporate resistance to several diseases, insects, and physiological defects into iceberg, romaine, and mixed lettuce cultivars and breeding lines adapted for coastal and low desert production. In the 2011-2012 period, major efforts targeted resistance to lettuce big vein disease, lettuce drop caused by *Sclerotinia species*, Verticillium wilt, lettuce dieback/tombusviruses, bacterial leaf spot, corky root, downy mildew, leafminer, lettuce aphid, tipburn, shelf-life of salad-cut lettuce, and multiple disease resistance. Minor programs addressed resistance to yellow spot. In all programs, horticultural traits, adaptation, and resistance to tipburn are essential.

Selections were taken from breeding populations and advanced breeding lines were evaluated as part of breeding for resistance to big vein disease, downy mildew, lettuce drop, Verticillium wilt, dieback, bacterial leaf spot, corky root, leafminer, tipburn, and pre-mature bolting.

Genetic studies concurrent with breeding programs are being conducted to determine the inheritance of resistance to big vein disease, bacterial leaf spot, dieback, leafminers, downy mildew, lettuce aphid, corky root, shelf-life of salad-cut lettuce, and Verticillium wilt. Publications during 2011-2012 included reports of original research on Verticillium wilt, downy mildew, leafminer, lettuce aphid, shelf-life, resistance to the fungicide triforine, and novel approaches to evaluate disease progress and combine phenotypic data from multiple trials.

**PROJECT REPORT
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PROJECT INVESTIGATORS: R. Hayes, I. Simko, B. Mou, J. D. McCreight, USDA/ARS,
Salinas

COOPERATING PERSONNEL:

R. Micheltore, O. Ochoa, M. Truco, - UC, Department of Plant Sciences, Davis, CA
G. Sandoya - UC, Department of Plant Sciences, Salinas, CA
K. Subbarao, P. Chitrampalam, K. Maruthachalam - UC, Plant Pathology, Salinas, CA
T. Gordon - UC, Plant Pathology, Davis, CA
S. Benzen, C. Bull, W. Wintermantel - USDA, Salinas, CA
S. Koike, R. Smith, M. Cahn, J. Bi - UC Cooperative Extension, Salinas, CA
M. Peralta - UA, Yuma, AZ
B. Maisonneuve - INRA, France
M. Matheron - University of Arizona Cooperative Extension, Yuma, AZ
P. Fashing, D. Milligan, J. Orozco, A. Atallah, J. Tanaka, M. Trent, M. Chawner - USDA,
Salinas, CA
Y. Luo, K. Williams, N. Garvey - USDA, Beltsville, MD
Growers, shippers, seedsmen- All districts

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 and B. Maisonneuve)

High level resistance to *Mirafiori lettuce big-vein virus*, the causal agent of big vein disease, is known in *L. virosa* accession (acc.) SAL012 (Hayes et al., 2008 Euphytica 164:493–500). We continued crosses to develop BC families from Salinas x SAL012.

A high level of partial resistance to big vein is available in the butterhead cultivar Margarita; we are introgressing this resistance into iceberg breeding lines adapted to early spring plantings in the Salinas Valley. Using January planted field experiments in Soledad, CA, we have developed 43 F_{3:4}, F_{4:5}, BCF_{3:4} iceberg types lines with reduced big vein symptoms.

We conducted an October planted low desert field experiment to develop big vein resistant icebergs for that region. The experiment includes 53 F₂ through F₅ families or breeding lines, as well as 83 accessions with potential for resistance to big vein. Fifty-four plants from 11 F₂ families and breeding lines with iceberg type characteristics and without big vein symptoms were selected. Seed of selected plants are being produced for next year's experiments.

b. Lettuce drop (with K. Subbarao)

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 compared to previous approaches. In 2011 we inbred 7 iceberg, leaf, and romaine populations to the F₃ through F₆ generation

We are introgressing resistance from the cultivar Eruption into romaine breeding lines. 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 type. Details on the resistance in 'Eruption' can be found in the publications: Hayes et al. 2010. HortScience, 45(3):333–341; and Hayes et al. 2010. Plant Breeding, 130:388-393. In a 2011 spring field experiment, we evaluated the lettuce drop resistance of 163 F_{4:5} lines from 'Darkland' x 'Eruption' and 'Hearts Delight' x 'Eruption'. All of these lines had been previously selected for resistance in the F₃ and F₄ generation. Ninety-five percent of these lines had lower disease incidence than 'Hearts Delight' and 'Darkland'. Fifteen lines were selected with high level resistance and good horticultural characteristics and were evaluated for resistance in a fall planted experiment with five replications. Five lines had significantly less disease than 'Green Forest', 'Hearts Delight', and 'Darkland'; all lines had significantly less disease than 'Darkland'. Core length was evaluated in this experiment to determine each lines propensity for early bolting; thirteen lines had shorter cores than 'Green Forest', 'Hearts Delight', and 'Darkland'.

From seven F4:5 families with resistance and good type, 44 F5:6 lines were developed by making single plant selections in a non-infested field experiment and will be used for additional evaluation.

We evaluated one romaine breeding line from ‘Balady Banha’ x ‘Darkland’; the lettuce drop resistance is from ‘Balady Banha’. Lettuce drop resistance has been consistently lower than the romaine cultivar Darkland. This breeding line was evaluated extensively for numerous quality and disease resistance characters in preparation for release. Additional details on the characters evaluated can be found in the section on advanced breeding line evaluations. The line is dark green with a closed top, hearting-type growth habit. The line has taller cores than most romaine cultivars and possess good levels of field resistance to downy mildew.

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

Two F₉ breeding lines were released in 2010 under the designation RH08-0472 and RH08-0475; additional details can be found in the publication: Hayes et al. 2011. HortScience, 46(3):501–504. We backcrossed RH08-0472 and RH08-0475 to Tiber and developed and additional nine F₆ resistant iceberg breeding lines for further evaluation in Verticillium infested grower fields. Crosses were made to breed iceberg lettuce combining race 1 Verticillium wilt resistance and corky root resistance; F₂ seed was produced and will be used in field experiments this summer. Our goal with this material is to develop and release early generation iceberg populations genetically fixed for resistance to corky root and race 1 isolates of *Verticillium dahliae*.

We are working to develop race 2 resistant breeding lines using the currently available sources of resistance. Using PIs 204707, 171674, 226641, and 169511, breeding populations from resistant × resistant and resistance × susceptible (commercial cultivars) crosses were produced. We continue to screen diverse accessions for resistance to race 2 isolates. Additional details can be found in genetics and germplasm evaluation sections of this report.

d. Fusarium wilt, a.k.a. root rot (with Fresh Express)

Diseased field was not available for planting in 2011. Two field tests are anticipated in 2012.

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 developing resistant cultivars is the only known protection against the disease. While modern iceberg cultivars are resistant to dieback, susceptibility is widespread in romaine and leaf-type lettuce.

In collaboration with R. Hayes, R. Sideman and K. Subbarao two romaine breeding-lines were developed and released into the public domain (Simko et al. 2010, HortScience). The SM09A and SM09B were selected based on their resistance to dieback, no premature bolting, overall phenotypic appearance, and slow deterioration after processing for salad. The SM09B was selected from a cross between Darkland and PI491224, while SM09A was developed from Green Towers × (Darkland × PI491224). Resistance to the disease in both breeding lines is derived from PI491224, a primitive romaine-type lettuce that is highly perishable when processed for salad. In replicated field trials, the two breeding lines showed complete resistance to dieback. Field observations were confirmed through the analysis of molecular markers closely linked to the dieback resistance gene *Tvr1* (Simko et al. 2009 - BMC Plant Biology, Simko et al. 2010 - Acta Horticulturae) Testing of salad-cut lettuce in modified atmosphere packaging indicates slower deterioration in the two breeding lines compared to other dieback-resistant romaines. Post-harvest deterioration of SM09A and SM09B is similar to that of Green Towers and Darkland, significantly slower than deterioration of PI491224 (Simko et al. in press – Crop Science).

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 shelf-life. Material from F₂ – F₄ generations was grown tested in field conditions for resistance to downy mildew, and tipburn. The best lines will be screened again 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, Simko et al. 2010 – Acta Horticulturae).

f. Bacterial leaf spot (with C. Bull)

We are introgressing resistance from Little Gem into iceberg type cultivars using a greenhouse testing method. This approach uses a limited amount of time and space, and facilitates the evaluation of a large number of progeny. In 2011, we evaluated 5796 seedlings from 93 BCF₂ iceberg type families in a greenhouse experiment where Xcv was sprayed on seedlings, and selected 207 disease free plants for further breeding.

We are refocusing the BLS resistance project towards developing resistance in cultivars suitable for spring mix. In 2011, two independent replicated greenhouse experiments were conducted to select two F₃ romaine type families from ‘Batavia Reine de Glaces’ (resistant cvr) x ‘Eruption’. The resistance in these families appears to equal in level and uniformity to ‘Batavia Reine de Glaces’. The families are variable for leaf color, shape, savoy, and margin serration.

g. Corky root (with C. Bull)

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. F₂ to F₆ 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.

Sixteen F₇ or F₈ 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 2011 for corky root resistance and horticultural traits. The corky root resistance of the breeding lines was similar to the resistant control ‘Glacier’, while their plant weight, core length, tipburn, and downy mildew 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 2011.

Genotype	Corky root ^z	Plant Wt. (g)	Core length (cm)	Tipburn leaves ^y
Cobham Green	7.9 A	422.8 D	5.1 AB	2.9 A
Bibb	7.8 A	396.4 D	4.9 B	0.3 B
Dark Green Boston	7.8 A	394.9 D	4.8 B	2.8 A
Margarita	7.7 A	434.6 D	4.1 B	0.3 B
Glacier	6.1 B	968.5 A	5.8 AB	0.0 B
MU10-479-1	5.2 C	637.0 BC	5.5 AB	1.1 AB
MU10-482	4.7 CD	535.3 CD	4.4 B	0.2 B
MU10-480-1	4.5 CD	741.5 B	7.1 A	1.7 AB
MU10-483	4.5 CD	539.0 CD	5.3 AB	0.0 B
MU10-481-1	4.3 D	509.3 CD	4.7 B	0.5 B

^z 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 downy mildew severity and head characteristics of red leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2011.

<u>Genotype</u>	<u>Corky root^z</u>	<u>Downy mildew</u>	<u>Plant Wt. (g)</u>	<u>Core length (cm)</u>	<u>Tipburn leaves^y</u>
Grand Rapids	8.0 A	1.8 DE	495.0 DEF	5.9 CD	1.8 AB
Prizehead	7.9 AB	3.0 BC	437.2 F	4.5 D	0.5 BC
Red Fox	7.8 ABC	3.1 BC	406.4 FGH	5.9 CD	0.0 C
Big Red	7.6 BC	3.3 ABC	420.2 FG	6.5 BCD	2.4 A
Lolla Rossa	7.6 BC	2.0 DE	285.7 H	10.5 B	0.0 C
Redina	7.5 C	1.4 E	393.2 FGH	15.6 A	0.0 C
Merlot	6.8 D	1.5 E	300.0 GH	6.8 BCD	0.0 C
Glacier	6.1 E	4.0 A	968.5 A	5.8 CD	0.0 C
MU10-393	5.9 E	2.5 CD	454.0 EF	4.1 D	0.0 C
MU09-493-1	5.9 E	2.5 CD	765.0 B	7.6 BCD	0.0 C
MU09-486-1	5.8 E	1.3 E	481.0 DEF	3.8 D	0.0 C
MU10-390	5.8 E	2.0 DE	580.0 CDE	5.0 CD	0.1 C
MU09-403-1	5.4 F	1.5 E	394.0 FGH	3.2 D	0.0 C

^z 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 2011.

<u>Genotype</u>	<u>Corky root^z</u>	<u>Plant Wt. (g)</u>	<u>Core length (cm)</u>	<u>Tipburn leaves^y</u>
Red Hot Cos	7.9 A	742.3 B	5.7 B	0.6 B
Red Eye Cos	7.7 A	648.7 B	6.7 B	0.6 B
Glacier	6.1 B	968.5 A	5.8 B	0.0 B
MU10-391-2	6.0 B	720.3 B	9.0 A	4.3 A

^z 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 (quantitative resistance) (with R. Michelmore, M. Truco, O. Ochoa, R. Antonise, M. Pel)

Downy mildew (caused by oomycete *Bremia lactucae*) is considered the most important disease affecting lettuce production. 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 slower rate of disease progress than on susceptible cultivars.

Five mapping populations have been being developed and currently tested in replicated field trials to detect quantitative trait loci for downy mildew resistance. Those populations originate from the crosses Salinas (susceptible) × Grand Rapids (resistant), PI491224 (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 already genotyped with SNP (R. Michelmore's laboratory) and AFLP (KeyGene, The Netherlands), while the Parade × Pavane population was genotyped with SNP markers only. Field-based testing confirmed presence of polygenes for resistance to downy mildew in all populations (Fig. 1). In the Grand Rapids × Iceberg population lines with both higher and lower level of resistance than either one of the two parental cultivars were identified. The Salinas 88 × La Brillante mapping population has been tested in seven trials. The most consistent QTLs were detected on linkage groups (LG) 2, 4, and 7. QTLs on LG 1 and LG 9 were detected in a single trial each. The Grand Rapids × Iceberg population has been tested in three trials. QTLs were detected on LG 2, LG 3, and LG 5. The Parade × Pavane population has been tested only once, and a single QTL was detected on LG 9.

Crosses were carried out to develop new breeding lines that would exploit field resistance observed in cvs. 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 with minimum number of lesions and non-bolting at the time of evaluation. The selected material is being evaluated for yield, size, uniformity, and tipburn resistance. Good level of resistance to downy mildew was observed in material originating from crosses with Balady Banha, Iceberg, and Grand Rapids.

Over 1,500 accessions were tested in replicated and unreplicated trials for their resistance to downy mildew. Among accessions tested in at least two trials the highest level of resistance was observed in 04G642 that harbors *Dm17* gene. This results show that *Dm17* still provides resistance against most of the downy mildew races detected in the Salinas-growing region. The highest level of polygenic resistance was recorded for Batavia-type cultivars Holborn Standard tested in 14 trials and Iceberg tested in 37 trials. High level of resistance was also observed in leaf-type cultivars Lolla Rossa and Grand Rapids (Simko et al. in press – Crop Science). Generally very high susceptibility to the disease was detected in iceberg and romaine type cultivars. This indicates that to achieve improvement in polygenic resistance to downy mildew, novel genes (or alleles) need to be introgressed into these types from other lettuce types or wild species.

In order to combine downy mildew disease progress data across multiple trials, we have developed approaches to calculate the area under the disease progress steps (Simko and Piepho, 2012 – Phytopathology). In addition we have developed statistical approaches to integrate

heterogeneous phenotypic data collected from multiple trials into the overall rating (Simko and Piepho, 2011 – Trends in Plant Science; Simko et al., in press – Crop Science).

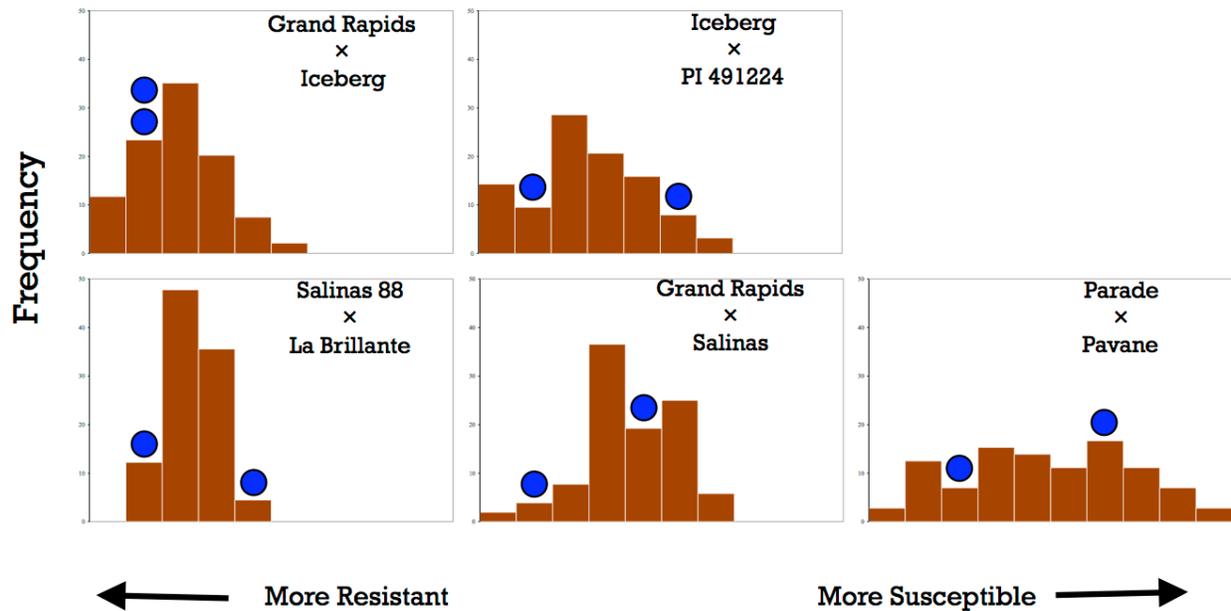


Figure 1. Distribution of resistance to downy mildew in five mapping populations. Blue circles indicate resistance observed in parents of the mapping populations.

2. Insect resistance

a. Leafminer (with Jianlong Bi)

Crosses were made 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. Crosses were also made among resistant sources to elevate the level of resistance.

F₂ to F₆ 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 resistance, and some of them were backcrossed to restore horticultural traits. Seven promising F₇ or F₈ breeding lines of green leaf, red leaf, and romaine lettuce were trialed at Spence Farm in Salinas from June to August 2011 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, core length, and tipburn of many lines were similar to or better than commercial cultivars (Tables 1-3). Some of these lines also showed resistance to corky root or downy mildew. These breeding lines will be evaluated again next year.

In collaboration with Dr. Jianlong Bi, we counted leafminer stings and mines as well as aphids on plants and conducted biochemical analyses (chlorophyll, carotenoids, phenolics, sugars, protein, etc.) for 16 leafminer resistant and susceptible genotypes grown in a growth chamber and the field to study the mechanism of leafminer/aphid resistance in lettuce. Green aphid density was correlated with protein and beta-carotene concentration in growth chamber and field (Table 4). Lettuce aphid density was associated with chicoric acid concentration in the field (Table 5). Leafminer stings at baby leaf stage were highly correlated with stings at mature plant stage (Table 6). Leafminer stings and mines were correlated positively with sucrose concentration of plants in growth chamber and negatively with concentrations of phenolics like flavonoids, chlorogenic acid, and rutin in lettuce plants in the field. Leafminer resistance was not associated with aphid resistance.

Table 1. 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 2011.

<u>Genotype^z</u>	<u>Stings/20 cm^{2,y}</u>	<u>Plant Wt. (g)</u>	<u>Core length (cm)</u>	<u>Downy mildew</u>
Two Star	7.0 A	570.7 BC	5.4 B	3.0 B
Waldman's Green	5.7 A	598.4 BC	17.3 A	3.6 A
Shining Star	4.3 B	651.0 B	8.9 B	3.8 A
MU06-857	1.1 C	502.3 BC	7.5 B	3.5 AB
MU10-607-1(<i>cor</i>)	0.9 C	642.5 BC	6.1 B	2.0 C
MU09-512-2	0.8 C	813.2 A	7.4 B	2.0 C
MU09-520-2(<i>cor</i>)	0.7 C	804.9 A	6.0 B	1.8 C
Grand Rapids	---	495.0 C	5.9 B	1.8 C

^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 $P < 0.05$.

Table 2. 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 2011.

<u>Genotype</u>	<u>Stings/20 cm^{2,z}</u>	<u>Plant Wt. (g)</u>	<u>Core length (cm)</u>	<u>Downy mildew</u>
Big Red	7.9 A	420.2 B	6.5 BC	3.3 A
Prizehead	6.1 B	437.2 B	4.5 C	3.0 AB
Red Fox	5.9 B	406.4 BC	5.9 BC	3.1 AB
Lolla Rossa	3.9 C	285.7 C	10.5 A	2.0 C
MU10-558	1.4 D	649.0 A	7.7 B	3.5 A
MU07-838	1.1 D	473.5 B	6.3 BC	2.3 BC

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

Table 3. 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 2011.

Genotype	Stings ^z /20 cm ^{2,z}	Plant Wt. (g)	Core length (cm)	Tipburn leaves
Green Forest	11.3 A	830.4 B	8.3 BCD	0.0 C
Evergreen	10.4 AB	1,116.0 A	12.5 A	7.9 A
Darkland EL	9.5 BC	697.4 B	7.2 CDE	0.0 C
Clemente	9.2 BC	762.0 B	6.3 DE	0.0 C
Heart's Delight	8.9 BC	1,165.0 A	9.0 BC	2.8 B
Short Guzmane	8.3 C	843.7 B	6.9 DE	0.3 C
Red Hot	5.1 D	262.0 C	5.3 E	0.9 C
Annapolis	4.5 D	280.0 C	9.4 B	0.0 C
MU09-462-1	0.9 E	1,067.0 A	7.3 CDE	0.0 C

^z Means in the same column followed by different letters indicate significant differences at $P < 0.05$.

Table 4. Phenotypic correlation coefficients of green aphids (*Myzus persicae*, *Macrosiphum euphorbiae* and *Aulacorthum solani*) per unit plant weight and concentrations of different phytochemical compounds for 16 leafminer resistant and susceptible genotypes grown in a growth chamber (G) and the field (F).

	Winged aphids/100g	Wingless aphids/100g	Total aphids/100g
Red aphids/100g	0.721**	0.566*	0.576*
G. Protein concentration	0.631**	0.890**	0.889**
G. Glucose concentration	- 0.561*	- 0.771**	- 0.771**
G. β -carotene concentration	0.567*	0.828**	0.826**
G. Lutein concentration	0.508*	0.819**	0.815**
G. Chlorophyll concentration	0.452	0.739**	0.735**
F. Protein concentration	0.596*	0.696**	0.699**
F. Chicoric acid concentration	0.649**	0.750**	0.753**
F. β -carotene concentration	0.293	0.636**	0.630**

* $P = 0.05$

** $P = 0.01$

Table 5. Phenotypic correlation coefficients of lettuce aphids (*Nasonovia ribisnigri*) per unit plant weight and concentrations of different phytochemical compounds for 16 leafminer resistant and susceptible genotypes grown in a growth chamber (G) and the field (F).

<u>Concentration</u>	<u>Winged aphids/100g</u>	<u>Wingless aphids/100g</u>	<u>Total aphids/100g</u>
G. Protein	0.283	0.516*	0.511*
G. Glucose	- 0.362	- 0.562*	- 0.558*
G. β -carotene	0.327	0.593*	0.587*
F. Protein	0.274	0.466	0.462
F. Chicoric acid	0.350	0.520*	0.517*

* $P = 0.05$
** $P = 0.01$

Table 6. Phenotypic correlation coefficients of leafminer sting/mine densities 30 & 65 days after planting (DAP) and concentrations of different phytochemical compounds for 16 leafminer resistant and susceptible genotypes grown in a growth chamber (G) and the field (F).

	<u>Leafminer stings/cm²</u>		<u>30 DAP mines/plant</u>
	<u>30 DAP</u>	<u>65 DAP</u>	
Leafminer sting/cm ² 30 DAP	----	0.923**	0.603*
Aphids/100 g plant	- 0.080	- 0.018	- 0.074
G. Sucrose concentration	0.657**	0.730**	0.790**
F. Flavonoid concentration	- 0.467	- 0.560*	- 0.700**
F. Chlorogenic acid	- 0.484	- 0.598*	- 0.594*
F. Rutin	- 0.379	- 0.491	- 0.603*

* $P = 0.05$
** $P = 0.01$

b. Lettuce aphid – Resistance to lettuce aphid (*Nasonovia ribisnigri*) biotype 0 (Nr:0) is controlled by a multiple allelic series of genes at a single locus. High-level (complete) resistance in *L. virosa* PIVT 280 is controlled by the single dominant gene Nr^C that was transferred to European cultivars and is being transferred to U.S. types by commercial breeders. Resistance in *L. virosa* PI 274378 is complete and allelic to resistance in PIVT 280. 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 subject to chance infestation, and in controlled-infestation field studies using whole plants and leaf cages. Seed of 59 CGN accessions reported resistant to Nr:0 and Nr:1 were obtained for testing against California strains of Nr:0 and characterization of their resistance phenotypes.

3. Adaptation and Quality

a. Adaptation to low desert environments

Four field experiments were conducted to breed slow bolting, big-vein resistant, and tipburn resistant lettuce for adaptation to fall, mid-winter, and late spring plantings in the Yuma Valley of AZ. Reports on breeding for slow bolting, resistance to big-vein and resistance tipburn can be found in those sections of this report.

b. Bolting resistance for fall plantings

We developed three F₇ iceberg breeding lines from the cross 87-714-8 x Autumn Gold with resistance to premature bolting for fall plantings in the low desert. We have completed evaluation of these lines for mid-September plantings as well as for diverse plantings. We are currently summarizing data in order to release these lines in late 2012.

Bolting resistance for fall plantings is also being developed in romaine germplasm. In a mid-september planted field experiment, bolting resistance was evaluated in 48 lettuce cultivars, 63 lettuce accessions, and 20 romaine type families. Thirty-three plants from five F₂ families and five F₄ or F₅ families with bolting resistance (short cores), romaine type plant architecture, and dense heads were selected. The sources of bolting resistance in these selections are the cultivars Siskyou, Tall Guzmaine, Blonde Lente a Monter, Medallion, Eruption, Valmaine, and Noga.

c. Tipburn

Three F₅ iceberg breeding lines from Salinas x Vanguard 75 were developed by Dr. Ed Ryder (USDA retired) via selection for the absence of tipburn symptoms and iceberg type head characteristics. We are currently summarizing data in order to release these lines in late 2012.

We are increasing our focus on developing improved tipburn resistance in romaine cultivars adapted to coastal and desert production using tipburn resistance found in modern iceberg cultivars. In multiple low desert and Salinas Valley field experiments in order to evaluate more than 200 families or recombinant inbred lines, we developed twelve F_{4.5} romaine breeding lines from 'Green Towers' x 'Salinas' with consistently lower tipburn symptoms than 'Green Towers'. In a December planted low desert field experiment using these breeding lines, we selected 34 plants from six families with improved horticultural characteristics.

d. Shelf-Life of Processed Lettuce & Spring Mix (with R. Michelmore)

The USDA has developed modified atmosphere packaging and controlled atmosphere chamber assays to detect genetic differences for shelf-life in salad-cut field-grown lettuce (Hayes and Liu, 2008. J. Amer. Soc. Hort. Sci. 133: 228–233). We currently use these methods in our breeding program to select against rapid decay while introgressing disease resistance from un-adapted sources. In 2011 Salinas Valley and low desert field experiments, we assessed the shelf-life of 16

advanced breeding lines in the dieback, downy mildew, tipburn, lettuce drop, and pre-mature bolting resistance breeding programs. Lines with poor shelf-life will not be released.

Understanding the genetics of shelf-life of processed lettuce in modified atmosphere packages may enable the efficient breeding of cultivars with industry acceptable shelf-life. Assessments using field grown plants of the recombinant inbred line (RIL) population Salinas 88 (good shelf-life) × La Brillante (poor shelf-life) was conducted between 2009 and 2011. Lettuce used for shelf-life evaluations originated from trials grown in Yuma, AZ (one trial in 2009/10) and Salinas, CA (three trials in 2010 and 2011), and were packaged using nitrogen flushed bags or air. Shelf-life data were used to determine the segregation of shelf-life, and to locate QTL for shelf-life on a genetic map of SNP markers from UC-Davis and AFLP markers provided by KeyGene. Three significant QTL were detected; one QTL on chr. 4 was detected in all experiments and explains 25-70% of the phenotypic variation depending on the experiment. Work was initiated to evaluate shelf-life in the other mapping population where a large difference was previously observed between the two parental cultivars (Parade – intermediate shelf life, and Pavane – poor shelf life).

We continue screening accessions from all types of cultivated lettuce to identify material with good and poor shelf-life. Up to this date we have screened 160 cultivars and advanced breeding lines for their performance. F₂ seed was produced to develop additional populations for genetic analysis.

B. Genetic studies

1. Bacterial Leaf Spot (with C. Bull)

We previously discovered a hypersensitive response (HR) in the cultivar Little Gem to Xcv (the bacteria that causes BLS). The HR response is a rapid leaf necrosis at the site of pathogen inoculation that is known to be indicative of disease resistance. In this research hypersensitive responses were detected by infiltrating a suspension of Xcv into the leaf tissue; lettuce genotypes with the HR develop necrosis in under 48 hours. In a single greenhouse experiment using a population of 93 recombinant inbred lines (RILs) derived from ‘Clemente’ x ‘Little Gem’, HR segregated as a single gene. To determine if the HR is an indicator of BLS resistance, we compared the results from a greenhouse inoculation where Xcv is sprayed on the lettuce leaves to the results of a HR infiltration test using 15 RILs from ‘Salinas 88’ x ‘Little Gem’. Six of the RILs expressed the HR while 9 did not, and there was a strong correlation between HR and lower disease severity. We also identified a HR to Xcv in the accessions La Brillante. In a greenhouse experiment, Xcv was sprayed on 91 RILs from ‘Salinas 88’ x ‘La Brillante’ to evaluate disease severity. This population has an existing molecular marker linkage map (Hayes et al., 2011, Theoretical and Applied Genetics, 123:509-517), which was used in quantitative trait loci analysis to identify a single large effect QTL on linkage group 2 for BLS resistance (low disease severity) inherited from ‘La Brillante’.

2. Big Vein (with R. Michelmore)

We are collaborating with UC-Davis to determine the inheritance of big vein resistance in *L. sativa*. Eighty F₆ recombinant inbred lines (RILs) from the cross Parade (susceptible) x Pavane (resistant) were developed by Ed Ryder, and were subsequently tested over three years for resistance in greenhouse experiments. Molecular marker genotyping and QTL analysis conducted by UC-Davis identified three QTL on linkage groups 3 and 4. Big vein incidence data was collected from a January planted field experiments and used for QTL analysis. A single small effect QTL on chromosome 4 was detected.

3. 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. The work was published in *Theoretical and Applied Genetics*, 2011, 123:509-517. We have developed F₂ populations from crossing 'La Brillante' with other sources of race 1 resistance. These populations are being tested in greenhouse experiments to determine the existence of resistance genes independent of *Vr1*.

Race 2: We are working to develop race 2 resistant breeding lines using the currently available sources of resistance. Using the partially resistant PIs 204707, 171674, 226641, and 169511, approximately 2400 F₃ families from 11 resistant × resistant and resistance × susceptible (commercial cultivars) crosses are being produced. We are on schedule to begin greenhouse and field testing in the fall of 2012 to determine the segregation of resistance and to select resistant families or crosses for more detailed study.

4. Use of triforine as a selectable marker for detecting hybrids (with R. Michelmore, M. Truco)

Lettuce is a highly inbred species with a compound autogamous floral structure that makes manual cross-hybridization less than 100% reliable. Therefore, accurate testing of F₁ hybrids is necessary to eliminate offspring resulting from self-pollination. We evaluated the potential of triforine as a selectable marker in breeding programs.

Some lettuce cultivars are highly sensitive to triforine, an inhibitor of sterol biosynthesis found in some commercial systemic fungicides. First symptoms of a sensitive reaction are usually observed within 24 to 48 hours after treatment and include severe wilting, necrosis and rapid plant death. We mapped a single dominant gene (*Tr*) that confers sensitivity of lettuce to triforine to linkage group 1 of the integrated genetic map of lettuce. The occurrence of sensitivity is not uniform across horticultural types of lettuce. While over 80% of green-romaine lettuce cultivars tested were sensitive, most cultivars of all other lettuce types were insensitive to triforine. All accessions of wild *Lactuca* spp. were insensitive to triforine. Allelism tests using F₁ and F₂ progeny revealed that sensitive cultivars of all horticultural types likely carry the same *Tr* gene. The dominant allele for sensitivity found in cultivated lettuce probably had a monophyletic origin. The reaction to triforine can be used as a marker for detecting hybrids originating from a

cross between phenotypically similar parents with different responses to triforine treatment. It also provides an indication of genotypes for which applications of triforine-containing fungicides are inappropriate.

Because all tested iceberg cultivars were insensitive to triforine, insensitivity to triforine cannot be used in this horticultural type as a phenotypic marker when testing success of pollination. However, triforine can be successfully used as a phenotypic marker for detecting hybrids between otherwise phenotypically similar green-romaine type cultivars, if they differ in their reaction to triforine. Though DNA-based molecular markers are informative for hybrid identification, the simple and inexpensive seedling or detached leaf triforine assay could be used in breeding programs when available. We compiled a list of 225 *Lactuca* accessions that were tested for their reaction to triforine. Detailed information can be obtained from I. Simko or accessed at Euphytica (Simko et al., 2011, Euphytica DOI 10.1007/s10681-011-0407-0).

C. Germplasm evaluation, maintenance and use

1. Screening

a. Verticillium wilt

Previous research identified two races of *V. dahliae* capable of causing disease in lettuce. La Brillante and other germplasm are resistant to Race 1; no sources of resistance to Race 2 are known. We are screening PIs for resistance to Race 2 by conducting unreplicated greenhouse experiments to identify candidate sources of resistance, which are then tested in replicated greenhouse experiments to confirm resistance. In all experiments, plants are assessed for disease symptoms after they have flowered, and asymptomatic plants are tested for *V. dahlia* colonization by plating stem sections on semi-selective NP10 media. Through 2011, we have screened 824 accessions using race 2 *V. dahliae* isolate VdLs17. Of these, 120 accessions are being treated as candidate sources of resistance that require further testing. Partial resistance has been confirmed in four accessions (PIs 169511, 171674, 204707, 226641) which are being used to develop populations for breeding and genetics research.

Several *Lactuca serriola* accessions collected in Armenia were identified by UC-Davis as potentially resistant to race 2 isolates. In collaboration with R. Michelmore and K. Subbarao, we have ongoing greenhouse experiments to evaluate resistance in this germplasm to race 2 isolates of *V. dahliae*.

b. Spring Mix Lettuce

Seventeen lettuce lines with unique phenotypes (e.g., leaf shape, coloration) were increased in the greenhouse for evaluation as potential components of Spring mixes. Five of eight lettuce mutants compared favorably with eight lettuce cultivars for leaf number in a Fall planting.

c. Wound induced leaf edge browning (WILE)

Chopped samples of 13 lettuce cultivars from two harvest dates were stored in normal atmospheres at 10°C (50°F) and evaluated for browning at 24-hr, 7-days, and 10-days. A rating scale (0 to 9) was developed for assessment of WILE. Differences in browning were observed within heads (upper half of head vs. bottom half) and among cultivars 24-hr and 10-days post-chopping.

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. In the 2011-2012 period, requests were made for Verticillium wilt resistant iceberg breeding lines (RH08-0472, RH08-0475) and dieback resistant romaine lines SM09A and SM09B.

Exploration and collection of wild *Lactuca* was sponsored through the USDA, Plant Exchange Office. In 2011, seed from 19 accessions of five wild *Lactuca* species were collected in the Russian Caucasus by Svetlana Litvinskaja and Ramazan Murtazaliev. Seed was distributed to UC-Davis for race 2 Verticillium wilt resistance screening, and are also being regenerated and characterized in Salinas, CA.

D. 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. 201. HortScience 46:501-504. 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 2011, we characterized nine romaine and seven iceberg breeding lines and cultivars for yield, horticultural characters, shelf-life of salad and arrival quality, and for resistance to big vein, dieback, Verticillium wilt, lettuce drop, downy mildew, tipburn and pre-mature bolting.

E. Recent publications relevant to this project

- Atallah ZK, Hayes RJ, Subbarao KV (2011): Fifteen years of *Verticillium* wilt of lettuce in America's salad bowl: A tale of immigration, subjugation, and abatement. *Plant Disease* 95:784-792.
- Hayes RJ, Maruthachalam K, Vallad GE, Klosterman SJ, Simko I, Luo Y, Subbarao KV (2011): Iceberg lettuce breeding lines with resistance to *Verticillium* wilt caused by race 1 isolates of *Verticillium dahliae*. *HortScience* 46:501-504.
- McCreight JD and Liu Y-B. 2012. Resistance to lettuce aphid (*Nasonovia ribisnigri*) biotype 0 in wild lettuce accessions PI 491093 and PI 274378. *HortScience* 47:179-184.
- Mou B 2011. Green leaf lettuce breeding lines with resistance to corky root, 06-831 and 06-833. *HortScience* 46: 1324-1325.
- Mou B 2011. Mutations in lettuce improvement. *International Journal of Plant Genomics*. Doi:10.1155/2011/723518.
- Simko I, Hayes RJ, Truco MJ, Michelmore RW (2011): Mapping a dominant negative mutation for triflorine sensitivity in lettuce and its use as a selectable marker for detecting hybrids. *Euphytica* 182:157-166.
- Simko I, Piepho HP (2011): Combining phenotypic data from ordinal rating scales in multiple plant experiments. *Trends in Plant Science* 16:235-237.
- Simko I, Piepho HP (2012): The area under the disease progress stairs: Calculation, advantage, and application. *Phytopathology* 102:381-389.
- Simko I, Hayes RJ, Kramer M (in press): Computing integrated ratings from heterogeneous phenotypic assessments: A case study of lettuce post-harvest quality and downy mildew resistance. *Crop Science* doi: 10.2135/cropsci2012.02.0111.