ABSTRACT
CALIFORNIA LETTUCE RESEARCH BOARD

for the period
(April 1, 2007-March 31, 2008)

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 desert production. In 2007, major efforts targeted resistance to lettuce big vein disease, lettuce drop / *Sclerotinia species*, Verticillium wilt, Fusarium root rot, lettuce dieback/tombusviruses, bacterial leaf spot, corky root, leafminer, lettuce aphid, tipburn and multiple disease resistance. Minor programs addressed increasing nutritional content, as well as resistance to powdery mildew and yellow spot. In all programs, horticultural traits, adaptation, and resistance to tipburn are essential.

In 2007, we confirmed resistance in previously identified germplasm to Verticillium wilt, Yellow Spot, Fusarium wilt and lettuce aphid. New candidate sources of resistance were identified to race 2 isolates of *Verticillium dahliae*. Selections were taken from breeding populations, and advanced breeding lines were evaluated, as part of breeding for resistance to big vein disease, lettuce drop, Verticillium wilt, powdery mildew, dieback, bacterial leaf spot, corky root, leafminer, tipburn, bolting, and for increased nutritional content.

Genetic studies concurrent with breeding programs are being conducted to determine the inheritance of resistance to big vein disease, lettuce mosaic virus, lettuce drop, leafminers, and Verticillium wilt. Publications during 2007-2008 included reports of original research on Verticillium wilt, lettuce shelf-life, leafminers, genetic analysis with association mapping, and a book chapter on lettuce breeding.

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COOPERATING PERSONNEL:

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P. Fashing, D. Milligan, J. Orozco, L. Lai, B. Robinson, J. Tanaka - USDA, Salinas, CA
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, 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)

Hybrid materials between *Lactuca virosa* accession IVT280 and several European cultivars provided by B. Maisonneuve, INRA, Montfavet, France have demonstrated a high level of partial resistance to big vein disease. Two related lines, 03-366-3M and 03-366-9M were selected for breeding, and we subsequently demonstrated the high likelihood that these breeding lines contain novel genes from *L. virosa* for big vein resistance (Hayes and Ryder, 2007, HortScience 42:35-39). We are using these parents to develop big vein resistant breeding lines that are genetically independent of Pacific. In 2007, we tested 407 $F_{5:6}$ breeding lines from 03-366-9 x Clemente, 03-366-9 x Salinas, and 03-366-9 x Salinas 88 in two replicated greenhouse experiments. Thirty-one percent of the families were more resistant than the *L. sativa* parent, and were selected for further breeding.

A high level of partial resistance to big vein is available in the cultivar Margarita and in a diversity of *L. sativa* PIs; we are introgressing this resistance into iceberg and romaine type breeding lines. Thirteen $F_{3:4}$ families from Margarita x Sniper and Margarita x Salinas 88 were developed by selecting for head type and resistance in field experiments in Soledad, CA. Seventeen plants were selected from big vein resistant families. An additional 20 F_2 plants from Sniper x Margarita were selected for head type and lack of big vein symptoms in field plantings in Soledad, CA and Salinas, CA. These lines will be evaluated in a 2008 Soledad, CA field experiment. Advanced lines from Pacific x (Pacific x (Salinas x PI 273589)) were tested for potential release. We determined in field experiments that these lines lacked sufficient resistance, as compared to Pacific, to warrant release. Two cos PIs, 278068 and 278078, were tested as a potential sources of resistance to improve romaine type cultivars. We determined that the resistance in these lines is no greater currently available commercial romaine cultivars. We will not pursue these two PIs any further.

An experiment was conducted in a Yuma, AZ commercial field to develop crisphead germplasm with big vein resistance for mid winter plantings in the Imperial and Yuma valleys. F_3, F_4, F_5, and F_7 breeding lines incorporating resistance from Pacific, Pavane, and Margarita were evaluated for type and resistance; 37 selections with improved head type were taken from families with low incidence of big vein. These experiments have been conducted in a grower-cooperator field in Yuma, AZ. Producing seed from plants selected in this field is logistically difficult. To overcome this problem, we are working with the University of California, Desert Research and Extension Center (DREC) in El Centro, CA to develop a big vein breeding nursery. To initiate this process, two box crates of lettuce roots from big vein symptomatic plants were taken from a nearby infested commercial field, and incorporated into a field plot at the DREC. The plot will be seeded with a susceptible cultivar in October 2008 to assess the disease pressure.

b. Lettuce drop (with K. Subbarao and B. Pryor)

We are developing resistant crisphead and romaine breeding lines using diverse germplasm. In 2007, the *S. minor* infested breeding nursery in Salinas, CA was fumigated to control weed and disease problems. Consequently, this site could not be used for a spring field experiment. A grower's field was used for a spring experiment instead, but did not develop sufficient disease to facilitate selection of resistant lines. A *S. minor* infested field was re-established at the USDA research facility in Salinas, CA in the fall of 2007, and an experiment was conducted to select resistance breeding lines. Sixty-five romaine and iceberg breeding lines and cultivars were tested, and extremely high disease pressure was observed (99% mortality in Batavia Reine des Glaces). Romaine and iceberg F_4 and F_5 breeding lines with resistance from Holborn Standard, Great Lakes 54, PI207490, PI177426, and PI176594 were selected for further testing.

Resistance from Latin type cultivars (Little Gem, Pavane, Eruption) is being pursued. In non-infested experiments, 31 F_2 plants were selected for head type to introgress resistance from Eruption and Little Gem into iceberg and romaine breeding lines. Additionally, ten new F_2 families were developed for breeding.

Sclerotinia sclerotiorum inoculated field experiments were conducted with cultivars, breeding lines, and PIs during the winters of 2004/2005, 2005/2006, and 2006/2007 to determine if germplasm resistant to *Sclerotinia minor* in the Salinas Valley is also resistant to *S. sclerotiorum* in Yuma, AZ. The experiment was inoculated by incorporating sclerotia of *S. sclerotiorum* into the bed prior to planting. Resistance was measured as the percent mortality due to lettuce drop at harvest maturity. Variation for resistance was observed among the tested lines, and the correlation between the years was significant. Several lines known to be resistant to *S. minor* in Salinas were also resistant to *S. sclerotiorum* in Yuma, AZ. These include, PI251246, Little Gem, Pavane, and Eruption. Additionally, the susceptible check Batavia Reine des Glaces was among the most susceptible lines in all experiments. Most importantly, breeding lines that were selected for reduced mortality in Salinas, CA *S. minor* infested field experiments, were consistently among the most resistance lines in the *S. sclerotiorum* infested experiments in Yuma, AZ. Therefore, it appears that a strategy of selecting for resistance to *S. minor* in Salinas can develop lines that are additionally resistant to *S. sclerotiorum* in the low desert. Based in these results, we are developing lettuce drop resistance for the low desert by 1) developing resistant breeding lines using *S. minor* infested field experiments in Salinas, CA, and 2) evaluating resistant lines for adaptation in low desert field experiments.

c. Verticillium wilt (with K. Subbarao and S. Klosterman)

Three Verticillium wilt resistant breeding lines were released. Lines RH05-0336, RH05-0339, and RH05-0340 are F_9 iceberg type lettuce breeding lines with resistance to Verticillium wilt caused by *Verticillium dahliae*. Other than Verticillium wilt, RH05-0336, RH05-0339, and RH05-0340 have not been characterized for their reaction to other lettuce pathogens or their propensity for physiological defects. Greenhouse grown seed was produced and made publically available, and 13 samples have been distributed nationally and internationally. It is requested that appropriate recognition be made if the breeding lines contribute to research or the development of new germplasm, breeding lines, or cultivars. Written requests should be sent to Dr. Ryan Hayes, USDA-ARS, 1636 E. Alisal St., Salinas, CA 93905.

Advanced breeding lines with Verticillium wilt resistance from La Brillante are being developed that combine, yield, quality, and resistance to other lettuce diseases. In 2007, 50 F_3, F_4, F_5, and F_6 breeding lines were evaluated in a *V. dahliae* infested field experiment with 3 replications. Disease pressure was moderate, with Salinas 88 having 52% root symptoms. Fifteen F_5 and F_6 lines were selected from the infested field experiment. Using data from previously conducted infested field experiments, we selected 13 F_5 and F_6 resistant lines from a non-infested field experiment located at the USDA research station. Two resistant breeding lines were backcrossed to Glacier, Mist Day and Tiber, and F_2 seed from these crosses were produced for further breeding.

Substantial effort was devoted to identifying variation for verticillium wilt resistance between and within the different lettuce types. Two *V. dahliae* infested field experiments and two inoculated greenhouse experiments were conducted on 123 iceberg, Batavia, green leaf, red leaf, latin, and romaine cultivars. Resistance to race 1 was identified in red leaf, romaine, latin, and Batavia cultivars (Hayes et al., 2007, Plant Disease 91:439 – 445). No resistance was identified to race 2 isolates. We are using several sources of race 1 resistance identified by this research to breed resistant romaine and iceberg cultivars. Seventy-nine selections were made from F_2, F_4 and F_5 families or breeding lines to incorporate resistance from Merlot, Little Gem, Annapolis, Defender, Pavane, Eruption, Infantry, and Barnwood Gem. Through selection for non-symptomatic plants in infested field experiments, were develop two resistant lines from Little Gem x Clemente. We backcrossed these lines to Clemente, and produced F_2 seed in the greenhouse for further breeding.

d. Powdery mildew

Powdery mildew resistant iceberg breeding lines adapted to mid-winter low desert plantings are being developed using the butterhead cultivars Big Boston and Soraya as sources of resistance. We previously developed resistant breeding lines from Winterhaven x Big Boston, which were crossed to the cultivars Coyote, Bubba, and Winterselect. In a single November planted field experiment in Yuma, AZ, we evaluated 19 F_3 families from these crosses, and selected 46 plants from 10 families for low powdery mildew severity and head type. From these same crosses, we also selected an additional 10 F_2 plants. Resistant breeding lines were developed from Salinas x Soraya, and were backcrossed to Coyote. The F_2 seed from these crosses is currently being increased and will be available for field experiments in 2008.

e. Fusarium root rot (with T. Gordon, S. Koike, M. Matheron, B. Platts, and B. Tickes)

Fusarium wilt is present in a few scattered fields in central coastal California and is spreading in Arizona. Though three physiological races of Fusarium are known in Japan; only race 1 is present in California and Arizona. Breeding line 97-0857 from Japan expressed high-level resistance in greenhouse and field tests. Five romaine cultivars previously identified as potential sources of resistance had significantly lower disease incidence than ‘Empire’ and ‘Salinas’, and did not differ from 97-0857.

f. Lettuce dieback (with R. Michelmore, M. Truco, O. Ochoa)

We continue to incorporate resistance from primitive lettuce (PI491209, PI491214, and PI491224) into romaine and leaf-type germplasm. In addition, resistance from cultivars Salinas (iceberg type) and Balady Banha (stem type) were also tested in different genetic backgrounds. Two field experiments were carried out in 2007 at ‘Carr Lake’ in Salinas, CA. Spring planting consisted of 80 breeding lines (F_3 to F_7), 40 cultivars and accessions, and 230 recombinant inbred lines (RILs) developed by UC Davis. The population of RILs was developed from a cross between resistant iceberg type cultivar Salinas, and susceptible romaine type cultivar Valmaine. The experiment was conducted in a randomized complete block design with two replications. Disease progress was evaluated weekly and the Area Under the Disease Progress Curve (AUDPC) was calculated for each tested material. Final data are reported as relative AUDPC score (rAUDPC), where 100% is the maximum reached by the most susceptible genotype. Summer planting consisted of 200 diverse cultivars and accessions, and 150 RILs from the Salinas x Valmaine population. Similarly as in the spring planting, the AUDPC was calculated for each material and is reported as rAUDPC.

In the spring planting, rAUDPC in the resistant controls ranged from 0% (Bandit, Ruby Ruffles, Skyway, Western Green, and PI491209) to 5% (Sharp Shooter). The rAUDPC values in the susceptible controls were from 23% (Heart’s Delight) to 85% (Gallega). Four cultivars showed intermediate level of resistance (Cavalry – 9%, Cobham Green – 12%, Merlot – 15%, and Little Gem – 16%) and could not be clearly classified. From 230 RILs tested in this planting, 110 RILs were classified as susceptible, 104 RILs were classified as resistant and 16 were in the intermediate group. This segregation indicates the presence of a single dominant gene in the mapping populations. Resistance observed in breeding lines was usually satisfactory and ranged from rAUDPC 0% to 10%. The only exceptions were four F_6 families from a cross between romaine type cultivar Darkland and stem type cultivar Balady Banha (rAUDCP from 52% to 81%). These lines were developed by R. Grube and R. Haynes as a part of the sclerotinia-resistance breeding program, and were tested in the field trail because Balady Banha is resistant to the lettuce dieback. Additional material developed by R. Haynes and tested for resistance to dieback were eight F_5 families from the tipburn-resistance program (TBR) and four F_6 families from the Verticillium resistance program (VertR). All 12 families showed high level of resistance (rAUDPC from 0% to 2% and from 2% to 7%, respectively). In the summer planting the most resistant genotypes were leaf-type accession PI177418 (rAUDCP of 0%) and iceberg type cultivar Salinas (rAUDPC of 1%). On the other hand, the most susceptible cultivars were romaine type Tall Guzmaine, and stem type Balady Aswan (rAUDPC of 95% and 98%, respectively). Resistance in the RILs was similar as in the spring planting ($r = 0.682$, $P < 0.0001$).

Based on the resistance screening, earliness of bolting, and overall phenotypic appearance in two trails, 135 plants from 68 families were selected to develop material for further evaluations.

g. Bacterial leaf spot (with C. Bull)

We developed inbred lines from Salinas 88 x (Salad Crisp x Iceberg). These lines have consistently demonstrated significantly better resistance than Salinas 88 in field and greenhouse experiments. In 2007, we increased seed of these lines in the greenhouse for subsequent characterization in field experiments.

We are introgressing resistance from Little Gem and Batavia Reine des Glacies into iceberg type cultivars. These two cultivars have higher levels of resistance than Salad Crisp and Iceberg (Bull et al., 2007, Plant Health Progress doi:10.1094/PHP-2007-0917-02-RS), and may lead to breeding lines with superior resistance. During 2007, other lettuce research groups have intensified their efforts with Batavia Reine des Glacies. While we will continue to work with resistance in this cultivar, we choose to focus more heavily in 2007 on breeding with Little Gem in order to not duplicate efforts with other breeding programs. We continue to select resistance families and seedlings with 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 2007, we evaluated 161 F_3 families in six replicated greenhouse experiments from Little Gem x Sniper and Salinas 88 x Little Gem. Disease free seedlings from resistant families were selected for further breeding.

h. 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 to be highly resistant to the disease. When grown in soil from Watsonville, PI 491239 and PI 273597c had lower corky root severity than cultivars with the *cor* resistant gene. We are studying the inheritance of the resistance in these PI lines. The resistance from these lines is also being incorporated into cultivated lettuces.

We continued to make crosses to transfer the 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.

Six green leaf, ten red leaf, and three butterhead F_7 lines were tested in a replicated field trial at the Spence Farm in Salinas this summer for corky root resistance and horticultural traits. The corky root resistance, head weight, head diameter, head height, tipburn, core length, and core diameter of the breeding lines were similar to control cultivars.

i. Yellow spot (with Richard Smith)

We continued to investigate the cause of “yellow spots” disorder of lettuce, especially on romaine lettuce, and identified some putative resistant and susceptible genotypes. We are making some crosses and selections for high levels of resistance to the disorder.

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, Lolla Rossa, Bibb, and Tom Thumb and good horticultural types Salinas, Salinas 88, Tiber, Prizehead, and Lobjoits were selected in the field, and some of them were backcrossed to restore horticultural traits. Some promising F_7 or F_8 breeding lines of crisphead, romaine, red leaf, and green leaf lettuce were trialed at Spence Farm in Salinas in summer 2007 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 commercial cultivars (Tables 1-4). These breeding lines will be evaluated again next year.

BC_1F_6 plants of crosses between butterhead type and IVT 280 (*L. virosa*) from INRA, France were transplanted in the field to select for resistance to mining. However, no plants showed significant resistance against mining.

b. Lettuce aphid (with Yong-Biao Liu)

High-level resistance to the lettuce aphid (*Nasonovia ribisnigri*) derived from the wild lettuce PIVT 280 (*Lactuca virosa*) is available in European cultivars and is being transferred to western U.S. types. Additional, unique sources of resistance are needed in the event of the emergence of a resistance-breaking strain of the lettuce aphid. In previous, controlled-infestation greenhouse tests, the level of aphid resistance in PI 274378 was comparable to that in PIVT 280, whereas the level of aphid resistance in PI 491093 was lower than in PIVT 280 and may be the same as the partial type aphid resistance described in *L. virosa* accession PIVT 273. In a replicated, controlled infestation field test, PI 491093 exhibited resistance comparable to PIVT 280 and 'Barcelona'.

3. Adaptation and Quality

a. Adaptation to low desert environments

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

b. Bolting resistance for fall plantings

High temperatures during fall plantings in the low desert region can result in premature bolting. We developed three F_7 iceberg breeding lines from the cross 87-714-8 x Autumn Gold. Each was developed by selecting for short cores and Autumn Gold head characteristics in mid-September planted field experiments in Yuma, AZ. These lines are similar to Autumn Gold in appearance, but with larger, heavier heads. They are less uniform than Autumn Gold, due to a tendency for protruding ribs. In mid-September plantings, the bolting resistance is equivalent to Empire. We are increasing seed of these lines for distribution to seed companies, but are delaying a formal release until sufficient seed quantities can be produced.

Bolting resistance for fall plantings is also being investigated in romaine germplasm. In two mid-September planted unreplicated field experiments in 2006 and 2007 located in Yuma, AZ, we evaluated core length in 24 romaine cultivars. Core lengths ranged from 6.5 cm (Siskyou) to 40.5 cm (BraveHeart) in 2006, and from 4 cm (Tall Guzmaine) to 18 cm (Triple Threat) in 2007 (Fig. 1). Crosses were made between PIC type romaine cultivars, and Siskyou, Tall Guzmaine, and Valmaine as sources of bolting resistance.

b. Nutritional studies (with M. Grusak)

Enhancing the nutritional levels of vegetables would improve the nutrient intake without requiring an increase in consumption. A breeding program to improve the nutritional quality of lettuce must start with an assessment of the existing genetic variation. To assess the genetic variability in mineral content, 52 genotypes including crisphead, leaf, romaine, butterhead, wild species, Latin, primitive, and stem lettuces were planted at the USDA field in Salinas in the summer and fall with four replications. Duplicate plant samples from each plot were dried at 70^{o}C for 48 hours and analyzed in collaborator Dr. Michael Grusak's lab by Inductively-Coupled Plasma Optical Emission Spectrometry (ICP-OES).

From previous screening experiments, we found that 'Greengo' and PI 206963 had high levels of β-carotene and lutein. They were crossed to different types of lettuce and selections were made in progenies to increase the carotenoid content of lettuce. A red leaf variety 'Merlot' was crossed to different lettuce types and selections were made in progenies to improve the anthocyanin content and antioxidant capacity of lettuce.

c. Tipburn

We are developing iceberg cultivars with high levels of tipburn resistance for spring harvests in the low desert. Sixty-seven F_5 and F_6 advanced breeding lines from the cross Salinas x Vanguard 75 were developed through a series of field experiments. These breeding lines and nine check cultivars were planted on December 18, 2007 in a single unreplicated field experiment at the Desert Research and Extension Center (DREC) in El Centro, CA. Head weight, head diameter, core height, and tipburn resistance were evaluated on April 8, 2008. The percentage of tipburn in Salinas 88 was 30%, in Gabilan, Tiber, and Heatmaster it was 40%, and in Dominquez 67 and Green Lightning the percentage of tipburn was 69%. Calicel had 100% tipburn, while we did not find any tipburn symptoms in Navajo. Tipburn resistance was found in a set of closely related breeding lines. Four F_5 and two F_6 Salinas x Vanguard 75 breeding lines had no tipburn;

all of these lines are derived from the same F_4 family. The head weight, diameter, and core height were similar to the check cultivars.

We are increasing our focus on developing improved tipburn resistance in mixed type cultivars adapted to coastal and desert production. We previously screened 66 green leaf, red leaf, romaine, and iceberg cultivars for tipburn resistance in mid-March planted field experiments in the Salinas Valley and in December planted field experiments Yuma, AZ. From this research we selected the most resistant romaine parents to initiate a breeding program to develop tipburn resistant romaine cultivars. However, initial breeding using remnant seed stocks of romaine x romaine crosses indicated that these crosses do not have sufficient genetic variation to select highly resistant romaine cultivars. Consequently, we have chosen to introgress tipburn resistance from iceberg type cultivars into romaine breeding lines. To initiate this effort, we evaluated tipburn on 500 F_2 plants from the cross Green Towers x Salinas in a December planted experiment in El Centro. Eighty-five selections were taken from this experiment, and seed is currently being increased. To generate additional material for breeding in coastal and desert environments, we made 24 crosses between six romaine cultivars (Clemente, Darkland, Green Towers, King Henry, PIC714, Valmaine) and four iceberg cultivars (Hallmark, Salinas, Salinas 88, Tiber) with high levels of tipburn resistance.

B. Genetic studies

1. Bacterial Leaf Spot (with C. Bull)

The inheritance of bacterial leaf spot from the cultivars Little Gem and Batavia Reine des Glacies is being investigated. In replicated experiments using randomly sampled or selected F_3 families derived from Little Gem, continuous and bell shaped distributions were observed. This suggests that resistance from Little Gem is conditioned by multiple genes. F_3 families from randomly selected F_2 plants were advanced for seed production to develop recombinant inbred line populations, and selected resistance families were increased for breeding purposes.

2. Big Vein (with R. Michelmore)

The inheritance of big vein resistance in *L. sativa* is not known. Eighty F_6 recombinant inbred lines (RILs) from the cross Parade (susceptible) x Pavane (resistant) were developed by Ed Ryder. We have accumulated 3 years of resistance data from greenhouse testing. In collaboration with UC-Davis, molecular marker genotyping was initiated in 2006 and was continued through 2007.

The inheritance of big vein resistance in *L. virosa* is unknown. Determining the number genes and their relationships will aid introgression of *L. virosa* resistance genes into lettuce cultivars. Because of sterility and other physiological abnormalities in hybrids between lettuce and *L. virosa*, intra-virosa families must be used in genetic studies with *L. virosa*. Through greenhouse testing in 2004 and 2005, we have identified resistance and susceptible *L. virosa* accessions. In 2007, the susceptible accessions bolted and flowered, and 4 crosses were made to accession IVT280.

3. Leafminer

To study the inheritance of leafminer sting density, parents, F_1, and F_2 plants of the crosses between *L. saligna* line PI 509525 (low sting density) and a butterhead cultivar 'Bibb' and stem lettuce 'Da Ye Wo Sun' (high sting density) were transplanted in the field, and the number of stings per unit leaf area was recorded. PI 509525 x 'Da Ye Wo Sun' F_1 plants had low leafminer sting density (Table 5), indicating that low sting density (resistance) was dominant. Chi-square analyses show that the segregation ratio of resistant and susceptible plants in the F_2 generation fits a single dominant gene model for resistance. Resistance to leafminer stings was also dominant in the F_1 generation of PI 509525 x 'Bibb' (Table 5), while the F_2 population segregated 3 resistant: 1 susceptible, indicating that resistance was controlled by a single dominant gene.

4. Lettuce mosaic virus (with R. Michelmore)

Investigations into a higher level of resistance in PI226514 indicated that the inheritance was due 2 recessive alleles, one of which is an allele of *mo1*. To test this model in a romaine background, we tested 53 F_3 families from (PI226514 x Salinas) x Clemente along with Salinas and Clemente. Analysis of this experiment is still in progress. We are currently testing a population of 54 F_6 recombinant inbred lines from Salinas 88 x PI226514tn in a growth room experiment. The data from this experiment will be used for genetic analysis, and to identify molecular markers linked to resistance genes.

5. Lettuce Drop. (with M. Truco, KeyGene, R. Grube, K. Bradford, R. Michelmore, A. van Deynze)

Dr. Becky Grube developed a recombinant inbred line (RIL) population for genetic analysis of resistance to lettuce drop from the early bolting oil seed lettuce PI251246. A population of 150 F_6 RILs from PI251246 x Salinas (lettuce drop susceptible) have been evaluated for resistance in Salinas, CA field experiments that were artificially infested with *Sclerotinia minor*. Each RIL was genotyped with AFLP markers in cooperation with KeyGene, and quantitative trait loci (QTL) analysis was conducted by M. Truco (UC-Davis). Four unlinked quantitative trait loci (QTL) were found for reduced lettuce drop incidence. Two of these QTL were at least linked to early bolting and/or low leaf area, and one of these appears to be from the susceptible parent Salinas. The other two QTL were of small effect and negligible significance. These findings indicate that resistance in PI251246 is likely conditioned from early bolting. Since an early bolting / resistance QTL was found in Salinas, these results also highlight the need for strict selection against early bolting when conducting lettuce drop resistance breeding with slow bolting parents.

6. Verticillium wilt (with R. Michelmore)

We previously determined that resistance to race 1 isolates in the cross Salinas 88 x La Brillante had segregation consistent with a single dominant gene in F_1, F_2 and RIL populations. To test this model further, we developed a population of Ms7-Salinas x (F_1 - Salinas 88 x La Brillante) for greenhouse testing. Since controlled pollinations in lettuce are difficult, the use of the male sterile Ms7-Salinas as a parent facilitates the production of large seed quantities. This population is expected to segregate 1 resistant : 1 susceptible.

The winter greenhouse experiment we conducted with this population did not develop sufficient disease to test this hypothesis (Glacier = 20% disease incidence; Ms7-Salinas = 38% disease incidence). It is likely that the winter growing conditions, and high losses of plants from Botrytis contributed to this outcome. We are developing additional populations to repeat this experiment.

Recombinant inbred lines (RILs) from Salinas 88 x La Brillante and Pacific x La Brillante are being developed to locate resistance gene(s) in the lettuce genome and the identify molecular markers suitable for marker assisted selection. $F_{5:6}$ seed lots were produced for both populations, and the Salinas 88 x La Brillante RILs were evaluated for resistance to race 1 isolate VdLs16 in a replicated greenhouse experiment using previously published methods (Hayes et al., 2007, Plant Disease 91:439 – 445). Segregation between the RILs fit a 1 resistant (complete absence of symptomatic plants) to 1 susceptible (at least one symptomatic plant) model for a single resistance gene ($\chi^2 = 1.3$; $p = 0.26$). DNA has been extracted from the Salinas 88 x La Brillante population, and was shipped to UC-Davis for molecular marker analysis, construction of a genetic linkage map, and identification of molecular markers linked to race 1 resistance. We produced $F_{5:6}$ seed lots of Pacific x La Brillante for greenhouse evaluation of race 1 resistance.

7. Population structure analysis with TRAP markers (with J. Hu)

The most common method for mapping genes in lettuce and is genetic linkage mapping. In contrast to genetic linkage mapping, association mapping is a method that detects relationships between phenotypic variation and genetic polymorphisms in existing cultivars, without the need for developing new mapping populations. The main drawback of association mapping is a possibility of false-positive results due to an unrecognized population structure. To assess population structure in lettuce, fifty-four lettuce cultivars representing five horticultural types together with six accessions from two wild species (*Lactuca saligna* L. and *Lactuca serriola* L.), were assayed for polymorphism with target region amplified polymorphism (TRAP) marker loci. The model-based clustering approach recognized three main subpopulations in cultivated lettuce that are well separated from wild species. Although the clustering based on molecular markers was generally in good agreement with horticultural types, some cultivars were classified differently or showed mixed origin. The effect of population structure on association mapping was tested on four traits with strong or weak correlation to the lettuce horticultural type and monogenic or polygenic mode of inheritance. Traits that were strongly correlated with lettuce types displayed many false-positive results when population structure was ignored, but the spurious associations disappeared when estimates of population structure or relative kinship (both based on molecular markers) were included in the statistical model. Use of horticultural types as a covariate was not sufficient to control for spurious associations in the monogenic trait with strong correlation to lettuce types. The best approach to avoid spurious associations in lettuce association studies is to assess relatedness of accessions with molecular markers and to include this information into the statistical model.

C. Germplasm evaluation, maintenance and use

1. Screening

a. Verticillium wilt

Research by G. Vallad 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 (Hayes et al., 2007, Plant Disease 91:439-445). We are screening PIs for resistance to Race 2 (isolate VdLs17) by conducting unreplicated greenhouse experiments to indentify 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. In 2007, we conducted an unreplicated greenhouse experiment with 167 PIs, 60 were selected for retesting in a replicated experiment. Thirty-eight PIs had been previously selected as candidate sources of resistance, and were tested in a replicated experiment in 2007. Twelve of these lines had significantly lower disease incidence than Salinas and La Brillante. The selected PIs will be re-tested in additional greenhouse experiments.

Other: We are continuing to screen PI materials for resistance to lettuce aphid.

2. Collection and distribution

We have distributed several hundred 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 2007, requests were made for Verticillium wilt resistant breeding lines RH05-0336, RH05-0339, and RH05-0340; dieback resistant romaine lines 01-778M, 01-781M, and 01-789M; 7 iceberg breeding lines that combine cork root and lettuce mosaic virus resistance; and breeding line 74-1076, a line developed by Ed Ryder as part of the lettuce drop resistance breeding program.

D. Field trials and cooperation

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 for our trials.

E. Recent publications relevant to this project

Hayes, R. J. and Liu, Y-B. 2008. Genetic variation for shelf-life of salad-cut lettuce in modified atmosphere packaging. Journal of the American Society of Horticultural Science 133:228-233.

Hayes, R.J., Vallad, G.E. and Subbarao, K.V. 2007. Inheritance of resistance to race 1 isolates of *Verticillium dahliae* in lettuce. HortScience 42:896.

Mou, B. 2008. Lettuce. in: J. Prohens and F. Nuez, ed., *Handbook of Plant Breeding,* *Vegetables I, Asteraceae, Brassicaceae, Chenopodicaceae, and Cucurbitaceae*. Springer, New York.

Mou, B., Hayes, R. J. and Ryder. E. J. 2007. Crisphead lettuce breeding lines with resistance to corky root and lettuce mosaic virus. HortScience 42(3): 701-703.

Mou, B. 2007. Inheritance of resistance to leafminers in lettuce. HortScience 42(4): 856.

Simko, I. and Hu, J. 2008 Population structure in cultivated lettuce and its impact on association mapping. Journal of the American Society for Horticultural Science 133: 61-68.

F. Appendix of Tables and Figures

Table 1. Mean values of leafminer sting density and head characteristics of crisphead lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2007.

Genotype	Stings[z]/20 cm^2	Head Wt. (g)	Core length (cm)	Tipburn leaves[y]
Premier	344.8 A	705.8 AB	4.9 B	0.5 B
Bronco	339.4 A	689.8 AB	4.4 B	0.0 B
Sniper	338.1 A	776.3 A	6.1 B	0.4 B
06-859-1	178.3 B	669.3 B	5.2 B	0.1 B
06-859-2	139.4 BC	639.5 B	5.4 B	0.5 B
06-902-1	130.5 C	623.8 B	5.4 B	0.8 B
06-840-1	111.0 C	487.5 C	11.2 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.

Table 2. 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 2007.

Genotype	Stings[z]/20 cm^2	Head Wt. (g)	Core length (cm)	Tipburn leaves[y]
Green Forest	337.4 A	909.0 BC	7.3 AB	0.0 C
Darkland	329.5 A	1,115.5 A	6.5 B	0.0 C
Heart's Delight	311.8 A	1,011.8 AB	7.2 AB	3.8 A
Clemente	223.3 B	1,016.0 AB	8.0 A	2.9 AB
06-896-1	134.9 C	746.3 C	8.0 A	0.9 BC

[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 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 2007.

Genotype	Stings[z]/20 cm^2	Head Wt. (g)	Core length (cm)
Big Red	154.9 A	505.8 A	8.8 A
Lolla Rossa	91.8 B	160.0 C	5.2 C
06-899-1	64.5 C	442.3 AB	7.4 B
06-895-1	45.6 C	359.0 B	5.7 C

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

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 2007.

Genotype[z]	Stings[y]/20 cm^2	Head Wt. (g)	Core length (cm)
Two Star	185.6 A	559.3 C	4.5 DE
Waldman's Green	121.0 B	565.0 C	8.9 A
Shining Star	96.1 C	477.3 C	5.2 CD
05-917-2	60.3 D	306.5 D	3.7 E
06-0857-1	52.8 DE	285.3 D	3.3 E
06-843-1 (*cor*)	35.0 EF	774.0 AB	6.2 BC
06-846-1 (*cor*)	34.0 EF	693.0 B	7.0 B
06-850-2	33.1 EF	250.0 D	3.2 E
06-844-1 (*cor*)	33.0 EF	811.5 A	6.7 B
06-841-1 (*cor*)	25.3 F	733.8 AB	6.5 BC

[z] Some breeding lines have the cor gene and are resistant to corky root.

[y] Means in the same column followed by different letters indicate significant differences at $P < 0.05$.

Table 5. Mean leafminer sting density of parents and F_1 generation, and chi square (χ^2) analysis of F_2 populations for their fit to a monogenic dominant inheritance model for resistance in two lettuce crosses evaluated in Salinas, California in 2007.

Genotype	Stings/cm^2 Mean ± S.D.[a]	Total plants	Resistant plants[b]	Susceptible plants[c]	χ^2 value	*P*-value
PI 509525	2.8 ± 1.5	40	40	0		
Da Ye Wo Sun	14.4 ± 1.9	40	0	40		
(PI 509525 x Da Ye Wo Sun)F_1	2.8 ± 0.7	23	23	0		
(PI 509525 x Da Ye Wo Sun)F_2	5.0 ± 4.6	252	193 (189)	59 (63)	0.26	0.50 -- 0.75
PI 509525	2.8 ± 1.5	40	40	0		
Bibb	11.5 ± 1.5	40	0	40		
(PI 509525 x Bibb)F_1	1.4 ± 0.8	48	48	0		
(PI 509525 x Bibb)F_2	4.7 ± 4.3	81	62 (61)	19 (20)	0.04	0.75 -- 0.90

[a] S.D., standard deviation. [b, c] Numbers in parentheses are expected values for a 3:1 segregation.

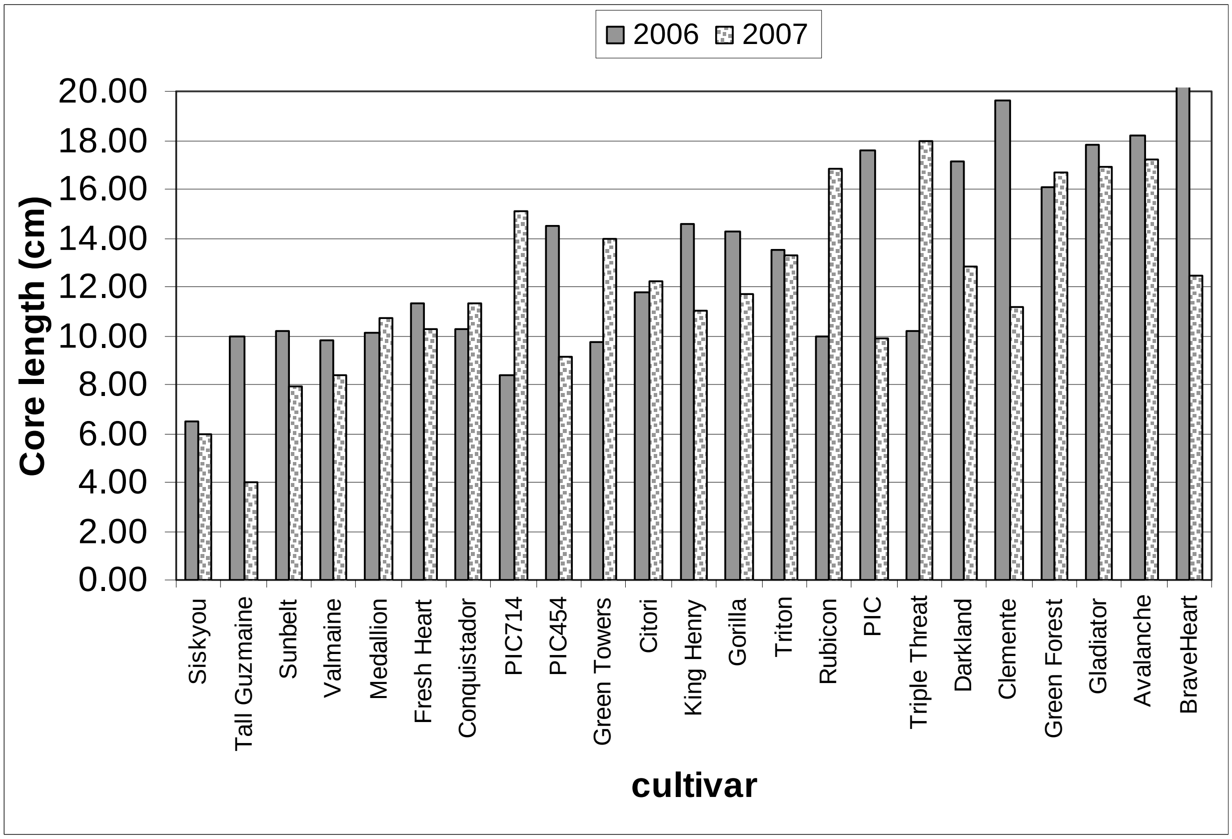

Fig 1. Core length for 23 romaine cultivars in unreplicated mid-September planted field experiments in Yuma, AZ in 2006 and 2007. Core length values are the average from measurements on 10 plants.