ABSTRACT
CALIFORNIA LETTUCE RESEARCH BOARD

for the period
(April 1, 2008-March 31, 2009)

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 2008, 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 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 Lettuce Drop, Verticillium wilt, Fusarium wilt, Yellow Spot, and lettuce aphid. New candidate sources of resistance were identified to race 2 isolates of *Verticillium dahliae* and pre-mature bolting. 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, 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, lettuce mosaic virus, leafminers, downy mildew, lettuce aphid, corky root, and Verticillium wilt. Publications during 2007-2008 included reports of original research on big vein, bacterial leaf spot, and development of EST-SSR markers for lettuce.

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COOPERATING PERSONNEL:

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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. Through four years of greenhouse testing, 102 $BCF_{5:7}$ lines were developed. These lines were planted in a field experiment in January 2009 that is currently in progress.

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. Thirty-three $F_{3:4}$ lines were developed by selecting for head type and resistance in field experiments in Soledad, CA. An additional 17 $F_{4:5}$ selected lines from Margarita x Sniper were backcrosses to Sniper or crossed to Salinas 88, and F_2 seed was produced.

A 2008 field 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.

b. Lettuce drop (with K. Subbarao)

We are initiating 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 than the current pedigree method, identifying resistant lines in 3 ½ years instead of 4 – 5 years as with the pedigree method. In 2008, F_3 families were developed for six different crosses. We will continue to inbreed these and additional families to the F_5 generation before we begin evaluating resistance.

We are developing resistant crisphead and romaine breeding lines using diverse sources of resistance. In 2008, a fall and spring infested field experiment was conducted to evaluate lettuce breeding lines and germplasm for lettuce drop resistance. Based on these experiments, we selected two iceberg lines from Salinas 88 x (75-501-1 x Holborn Standard) with disease incidences of 29 and 42%. This level of disease was significantly less than Salinas (87%) and Glacier (100%). One line was advanced for further evaluation of horticultural characters to determine its suitability for release; the other line is not suitable for commercial production. We have backcrossed both lines to adapted cultivars to develop populations with resistance and improved head type. We evaluated 36 inbred romaine breeding lines for lettuce drop resistance. Twelve lines were selected for re-evaluation of resistance, four lines were selected for testing of resistance and horticultural type, two lines were crossed to Green Towers to generate additional breeding populations.

We are working with new sources of resistance found in the small statured Latin type cultivars Eruption, Pavane, and Little Gem. These cultivars are not early bolting and have demonstrated resistance in repeated *S. minor* infested field experiments. However, their small size and upright growth habit may simply facilitate escape by promoting soil drying around the crown of the plant. In 2007 and 2008 we identified a single recessive allele in Latin x romaine crosses affecting plant height. From this work, we developed 29 F_3 families from four crosses to determine the effect of short or tall stature on resistance in *S. minor* inoculated field experiments. Based on a single fall field experiment, we found no difference in lettuce drop resistance among short, segregating, and tall F_3 families. This indicates that the single recessive gene for short stature was not sufficient to confer resistance, and development of tall statured romaine type breeding lines from these populations may be feasible. For 2009, we will repeat this experiment using an additional 96 F_3 families. These families will be grown concurrently at Spence farm to select for romaine type plants within resistant families.

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

Advanced breeding lines with Verticillium wilt resistance from La Brillante are being developed that combine yield, quality, and resistance to other lettuce diseases. Due to the unavailability of infested field locations, no field experiments to determine Verticillium wilt resistance were conducted in 2008. We have recently completed establishment of a race 1 infested field site at the USDA station, and we will commence field testing of our breeding material in 2009. Non-infested field experiments were conducted in 2008 using breeding lines and families previously determined to be resistant in infested field experiments. Twenty-one F_6 and F_7 resistant lines were evaluated in Soledad and Salinas field experiments for head weight, core height, diameter, maturity, tipburn resistance, big vein resistance, and shelf-life. Based on these data, nine breeding lines were advanced for further testing in 2009. F_2 seed was generated from backcrossing two resistant breeding lines to Glacier, Mist Day and Tiber, and we developed 35 F_3 families by selecting F_2 plants for head type in non-infested field experiments.

Substantial effort was devoted to identifying variation for Verticillium wilt resistance between and within the different lettuce types. Resistance to race 1 was identified in red leaf, romaine, Latin, and Batavia cultivars (Hayes et al., 2007, Plant Disease 91:439 – 445). We are using several sources of race 1 resistance identified by this research to breed resistant romaine and leaf cultivars. By selecting for head type in non infested field experiments in 2008, we developed 151 F_3, 74 F_4, and 12 F_5 families or lines incorporating resistance from Merlot, Annapolis, Defender, Pavane, Eruption, and Infantry. Through selection for non-symptomatic plants in infested field experiments, we previously developed a resistant romaine line from Little Gem x Clemente. We backcrossed this line 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 2009.

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

The Japanese, semi-iceberg lettuce ‘Costa Rica No. 4’ and ‘Salinas’ are comparable for resistance in greenhouse tests in Salinas, but the level of resistance is insufficient for the early fall desert plantings when the daytime temperature exceeds 100 F. A group of romaine cultivars previously identified to have levels of resistance potentially useful for the early desert plantings were crossed with susceptible and resistant cultivars (Table 1). Additional crosses will be made; F_2 populations will be developed.

f. Lettuce dieback

The lettuce dieback disease is caused by soilborne viruses of the family *Tombusviridae*. Resistance to the disease is high in iceberg-type cultivars, but is very limited in leaf lettuces and almost non-exist in modern romaine type cultivars. USDA-ARS previously released three romaine breeding lines with resistance to the disease. However, recent evaluations have shown that all three breeding lines and most of the romaine accessions bearing the *Tvr1* resistance gene have a very short shelf-life after processing into salad. Therefore, all advanced breeding lines are being assessed for both dieback resistance and shelf-life. A field experiment was carried out at ‘Carr Lake’ in Salinas, CA. The planting consisted of 222 breeding lines (F_2 to F_8), cultivars and plant introductions, and 114 recombinant inbred lines (RILs) developed by UC Davis (R. Michelmore’s laboratory). The population of RILs was developed from a cross between dieback resistant iceberg type cultivar Salinas, and susceptible romaine type cultivar Valmaine. The experiment was conducted in a randomized complete block design with two replications. Plants were checked weekly for disease symptoms in order to discriminate between plants dying due to dieback and unrelated causes. The percentage of plants that showed typical dieback symptoms (or were dead due to dieback) was recorded at harvest maturity. Resistant controls had very few symptomatic or dead plants at the time of harvest and the values ranged from 0% (most of the control material) to 4%. Susceptible controls ranged from

55% to 94%. Based on the resistance screening, earliness of bolting, and overall phenotypic appearance, 121 plants from 43 families were selected to develop material for further evaluations.

Shelf-life was evaluated as percent decayed tissue at the time point when the poor-quality control (cv. La Brillante) reached 100% decayed. The average decay in the material that is resistant to dieback was 90.7%, while cultivars susceptible to the disease showed average decay of 73.9%. The values for seven tested breeding lines, that carry the *Tvr1* gene was 62.3%. The slowest decay was observed in lines Sx08-006 and Sx08-003 with 41.1% and 43.6% decayed tissue, respectively. Another two breeding lines, Sx08-008 (52.5%) and Sx08-005 (54.6%) also showed a substantial improvement in self-life in comparison to the dieback resistant controls. The material will be tested this year again for shelf-life, resistance to biotic and abiotic factors (Verticillium wilt, lettuce drop, downy mildew, tipburn) and multiple horticultural characteristics. If the shelf-life and resistance results from last year are confirmed, the best breeding lines will be released.

g. Bacterial leaf spot (with C. Bull)

We released six $F_{6:8}$ (RH07-0370M, RH07-0373M, RH07-0379M, RH07-0380M, RH07-0386M, and RH07-0387M) and one $F_{4:5}$ (RH04-0157-3) iceberg breeding lines of lettuce (*Lactuca sativa* L.) with resistance to bacterial leaf spot (BLS) caused by *Xanthomonas campestris* pv. *vitians*. A copy of the full release statement can be found at the California Leafy Greens Research Programs website http://www.calgreens.org/abstracts.html. These breeding lines were selected from the cross (Saladcrisp x Iceberg) x Salinas 88. In replicated field and greenhouse testing, the level of resistance to BLS in these lines was significantly greater than Salinas 88 and equivalent to or better than Saladcrisp and Iceberg. The new breeding lines have partially covered heads with medium-dark-green and crisp textured leaves; leaf margins have variable degrees of leaf margin serration. Maturity is variable among the lines, but all are later than Saladcrisp. The seeds of each line are black in color. All breeding lines are susceptible to *Lettuce mosaic virus*. These breeding lines are the first western shipping type iceberg lettuces with resistance to BLS. They should be used as parents for further development of BLS resistant cultivars, or selected for adaptation to specific production environments.

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 Saladcrisp 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. Starting in 2007, other lettuce research groups intensified their efforts with Batavia Reine des Glacies. While we will continue to work with resistance in this cultivar, we have chosen to focus more heavily on breeding with Little Gem in order to not duplicate efforts. We continue to select resistant 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 2008, we evaluated and selected F_3 and F_5 lines from Little Gem x Sniper or Batavia Reine des Glacies. Inbred lines from Little Gem x Sniper were crossed to Sniper and Pacific, and F_2 seed is currently being produced.

h. Corky root (with C. Bull)

We 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. PI 491239 and PI 273597c had lower corky root severity than cultivars with the *cor* resistant gene in soil from Watsonville containing a potentially new corky root pathogen. We are studying the inheritance of the resistance in these PI lines. The resistance from these lines is also being incorporated into cultivated lettuce.

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.

Twenty-two F_7 or F_8 green leaf, red leaf, and butterhead breeding lines were tested in a replicated field trial at the Spence Farm in Salinas in summer 2008 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 resistance were comparable or better than control cultivars (Tables 2, 3 and 4).

i. Yellow spot (with Richard Smith)

We continued to study "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 level resistance to the disorder.

j. Downy mildew (quantitative resistance)

Downy mildew (caused by oomycete *Bremia lactucae*) is considered the most important disease affecting lettuce production. A large number of 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 is usually infected with the pathogen, but there are fewer and smaller lesions on fewer affected leaves, and a slower rate of disease progression than on susceptible cultivars.

Thirty selections in F_3 to F_6 families were made from crosses between susceptible material and donors of quantitative resistance. The selections were made from spring and summer plantings in Salinas. Plants were selected with low lesion numbers and lack of bolting at the time of evaluation. Two advanced breeding lines (Sx08-013 & Sx08-014) from our iceberg breeding program consistently showed lower levels of infection as compared to other iceberg material. They are being evaluated for yield, size, uniformity, and tipburn resistance. Three segregating populations are being developed to map quantitative trait loci (QTL) for resistance to downy mildew. These populations originate from crosses between cvs. Salinas (susceptible) x Grand Rapids (resistant), PI491224 (susceptible) x Iceberg (resistant), and Grand Rapids x Iceberg. Field testing confirmed the presence of polygenes for resistance to downy mildew in all three populations. In the Grand Rapids x Iceberg population, several lines with higher or lower levels of resistance than the parents were identified. This observation suggests that at least some of resistance loci are different between the two cultivars. Mapping resistance QTLs will be performed in collaboration with R. Michelmore's laboratory (UC Davis).

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. Nine 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 2008 with four replications, along with control 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 5, 6, 7, and 8). These breeding lines will be evaluated again next year.

b. Lettuce aphid (with Yong-Biao Liu)

High-level or complete resistance to the lettuce aphid (*Nasonovia ribis-nigri*) found in IVT280, an accession of *Lactuca virosa* and a distant relative of lettuce, is being transferred commercially to U.S. lettuce types. New sources of resistance will be necessary in the event the lettuce aphid overcomes this resistance. More than 1200 lettuce lines have been screened for resistance to lettuce aphid. Resistance in PI 491093 (*L. serriola*), initially comparable to IVT 280, is partial.

The partial resistance expressed by PI 491093 was further tested in greenhouse and field tests. In two greenhouse tests, individual plants of resistant (IVT 280, *Lactuca virosa*; 'Barcelona'; PI 491093) and susceptible (Salinas; PI 273597c, *L. serriola*) were each infested with a single, 24 to 48-hr old nymph. Differences in mean number of aphids per plant were apparent 14 days post-infestation (dpi) and more pronounced 21 dpi. The susceptible lines had many more aphids than the partially resistant PI 491093, which had more aphids than the lines with complete resistance (Fig. 1A). The differences in mean number of aphids per plant between the partially resistant PI 491093 and the entries with complete resistance diminished over time (Fig. 1A). In a third greenhouse test, five aphids were placed on seedlings of 'Salinas', IVT 280 and PI 491093. Mean number of aphids on IVT 280 was 6.3 compared with 44.0 on PI 491093 and 154.0 on 'Salinas' at 23 dpi (Fig.

1B). The entries were growing in the same cages and their leaves were overlapping, which may have enabled aphid movement from 'Salinas' to the other entries. To test this, the entries were then placed in separate cages for 25 days. Numbers of aphids decreased on the entries by 27 dpi and continued to do so on IVT 280 and PI 491093 through 48 dpi, but increased on 'Salinas' through 36 dpi before dropping (Fig. 1B).

PI 491093 again expressed partial resistance in two field tests. In one of the field tests, plants were transplanted and covered with aphid-proof fabric and infested on one of four dates with five 24-hr to 48-hr old nymphs. Numbers of aphids were counted about 30 dpi (Fig. 2A). In the second test, the entries were directly seeded in a field with other lettuce breeding lines and cultivars. Plants were randomly sampled near maturity and numbers of aphids were counted (Fig. 2B).

Frequency distributions of numbers of plants with different numbers of aphids 27 dpi (Fig. 3) show the phenotypic variation possible in expression of complete resistance in (IVT 280 and PI 274378D, both *L. virosa*), partial resistance (PI 491093, L. *serriola*; and PI 274375, *L. virosa*), and susceptibility ('Salinas') under greenhouse conditions favorable for aphid reproduction. Several F_1 progenies from crosses of IVT 280 with PI 274375 and PI 274378D showed similar variation in their expression of resistance (Fig. 3).

3. Adaptation and Quality

a. Adaptation to low desert environments

Three field experiments were conducted to breed bolting and tipburn resistant lettuce for adaptation to fall and late spring plantings in the Imperial Valley of CA or the Yuma Valley of AZ. Reports on breeding for resistance to 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 increased seed of these lines for further testing.

Bolting resistance for fall plantings is also being investigated in romaine germplasm. F_2 families were generated to move bolting resistance from Siskyou, Tall Guzmaine, and Valmaine into PIC type romaines. In unreplicated experiments, we continued to assess and select additional sources of bolting resistance for breeding.

c. Tipburn

Five F_5 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. These lines are also resistant to *Lettuce mosaic virus* (LMV). Field experiments with three replications were conducted with these lines at the Desert REC over two growing seasons (2007-2008, and 2008-2009) to compare tipburn incidence, core height, head weight and diameter to commercial cultivars. An additional assessment of only tipburn incidence was conducted in a 2006-2007 field experiment. All experiments were planted in mid December and evaluated in mid-April, when high temperatures insure that tipburn will occur. The breeding lines tended to have heavier, larger diameter heads with taller cores than the control cultivars, although these differences were not significant. The tipburn incidence across three field experiments for four breeding lines was equivalent or lower (38%, 24%, 38%, and 18%) than the commercial cultivars (Gabilan = 36%, Salinas = 52%, Tiber = 47%). One breeding line had high tipburn incidence (64%), as did the susceptible control Calicel (89%). Four of these breeding lines were similar to Salinas and Tiber in appearance with tipburn resistance equivalent to Gabilan, a normally lower yielding cultivar. Consequently, these lines appear to have commercial promise. The planting date used for these experiments is intentionally late in the growing season, in order to reliably evaluate tipburn resistance. However, it is atypical for commercial production, and further testing is needed in grower fields during more typical production times.

We are increasing our focus on developing improved tipburn resistance in mixed type cultivars adapted to coastal and desert production. Initial breeding using 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. In a December 2007 planted field experiment, approximately 500 F_2 plants were grown from the cross Green Towers (susceptible romaine) x Salinas (resistant iceberg) and individual plants were selected with romaine head characteristics and lack of tipburn symptoms. Tipburn incidence in the parents Green Towers (91%) and Salinas (30%) were high, and indicate that the environment was conducive for tipburn development. Thirty-eight plants were selected, grown to flowering, allowed to self-pollinate, and seed was harvested at the Desert REC. During the 2008-2009 growing season, the resulting F_3 families were planted in December for further selection of low tipburn incidence and romaine head characteristics. The tipburn incidence in the parents was lower than in the previous year (Green Towers = 47%, Salinas = 13%), and 169 tipburn free plants were selected. These plants are being allowed to set seed from self-pollination at the Desert REC; seed set is expected to be complete by mid-summer. In a Salinas Valley field experiment, 600 F_2 plants from Green Towers x Salinas were grown and 27 plants were selected for lack of tipburn symptoms and for romaine head type. The resulting F_3 families will be evaluated for tipburn resistance in a 2009 field experiment. To generate additional material for breeding in coastal and desert environments, F_2 seed was generated from 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 cultivar Little Gem 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. Recombinant inbred line populations from Salinas x Little Gem and Clemente x Little Gem are being developed, and were advanced to the F_5 and F_4 generation respectively.

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. Using this population, the USDA subsequently conducted greenhouse experiments over three years and UC-Davis generated marker data for 41 molecular markers. Single marker association analysis was conducted by UC-Davis, and two markers were significantly associated with resistance in 2 greenhouse experiments, while three markers were associated with resistance in a single greenhouse experiment. Additional molecular marker analysis is being conducted by UC-Davis to develop a full genetic map for this population.

The inheritance of big vein resistance in *L. virosa* is unknown. Determining the number of 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 resistant and susceptible *L. virosa* accessions. In 2008, crosses between susceptible accessions and IVT280 were grown for production of F_2 seed.

3. Leafminer

To study the inheritance of leafminer sting density and downy mildew resistance, six F_3 families from crosses between a *L. saligna* line PI 509525 (low sting density, downy mildew resistant) and cultivar 'Da Ye Wo Sun' (high sting density, downy mildew susceptible) were transplanted in the field in the fall 2008, and the sting density and downy mildew severity on each plant were recorded. The segregation of F_3 plants was consistent with a single dominant gene model for resistance to leafminer stings and downy mildew (Tables 9 and 10). Plant tissue was also collected from each plant for DNA isolation and genotyping. Some tentative SNP markers for sting density were identified in a BSA analysis of the (PI 509525 x Bibb) F_2 population.

4. Lettuce mosaic virus (with R. Michelmore)

Investigations into a higher level of resistance in PI226514 indicated that the inheritance was due to two recessive alleles, one of which is an allele of *mo1*. We tested a population of 54 F_6 recombinant inbred lines from Salinas 88 x PI226514tn in a growth room experiment, and data from this experiment are currently being analyzed.

5. 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. Recombinant inbred lines (RILs) from Salinas 88 x La Brillante and Pacific x La Brillante were developed to locate resistance gene(s) in the lettuce genome and to identify molecular markers suitable for marker assisted selection. $F_{5:6}$ seed lots have been 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 ($\chi^2 = 1.3$; $p = 0.26$). Molecular marker analysis, development of a genetic map, and QTL analysis was conducted by UC-Davis. A single significant QTL was located on linkage group 5; additional marker analysis is being conducted to place more markers in this region of the lettuce genome. We are currently testing the Pacific x La Brillante RILs in greenhouse experiments.

Substantial effort was devoted to identifying variation for Verticillium wilt resistance between and within the different lettuce types. Resistance to race 1 was identified in a diverse set of red leaf, romaine, Latin, and Batavia cultivars (Hayes et al., 2007, Plant Disease 91:439 – 445). Determining which cultivars have novel genes for resistance will reduce redundancy in the breeding program. Over the last few years, we have been generating two types of populations, F_2 families from resistant x resistant crosses and populations developed by crossing *Male sterile-7* Salinas by F_1 hybrids derived from resistant x resistant crosses. Both types of populations will be tested in greenhouse experiments to determine the presence and pattern of segregation for race 1 resistance.

6. Marker-assisted selection for dieback resistance (with L. McHale, M. Truco, O. Ochoa, R. Michelmore, and B. Scheffler)

A single dominant gene on chromosome 2 (*Tvr1*) was found to be responsible for the dieback resistance observed in modern iceberg lettuces. The population of 192 F_8 RILs developed from a cross between the susceptible romaine cultivar Valmaine and the resistant iceberg cultivar Salinas was used to pinpoint the position of the resistance gene with EST-based molecular markers. Nine markers closely linked to the *Tvr1* gene were subsequently tested for an association with the resistant phenotype in a set of 68 accessions from all horticultural types of lettuce. Sequencing of the marker that showed the exact fit with resistance revealed the presence of three haplotypes, two of them associated with the disease resistance. The High-resolution DNA melting approach that allows for the detection of all three haplotypes in a single analysis was successfully applied to study the marker-trait association in another set of 130 diverse accessions. The results confirmed the usefulness of the marker for marker-assisted selection in all types of cultivated lettuce. We are validating the marker-trait association on another set of accessions with a diverse genetic background. If confirmed, the marker information will be released to all interested parties (for more information contact Ivan Simko).

7. Development of EST-SSR markers

Microsatellites or simple sequence repeats (SSRs) are short, tandemly repeated motifs of DNA ubiquitous in all analyzed eukaryotic genomes. SSR-based molecular makers are frequently used in plant genetics due to their high reproducibility, codominant inheritance, and high information content. Though SSRs are markers of choice in many plant species, only a very limited number of SSR markers are publicly available for lettuce. The use of microsatellites has been limited in plants by the costs involved in isolating large numbers from the target species. *In silico* mining of SSRs from sequence databases provides an attractive alternative to the molecular approaches. Not only is the *in silico* approach time and cost effective but also it allows for the discovery of SSRs from expressed sequence tags (ESTs) that represent the coding region of the genome. EST-SSR markers are thus potential candidates for gene tagging and comparative studies in related species. A set of 61 simple sequence repeat (SSR) markers was developed from the 19,523 *Lactuca sativa* and *Lactuca serriola* unigenes. Assessment of population structure among 90 *L. sativa* cultivars with SSRs was in good agreement with classification into the horticultural types. The average marker heterozygosity was smallest in iceberg (0.097), Latin (0.140), and romaine-type (0.151) cultivars while highest in leaf (green leaf 0.208 and red leaf 0.240) lettuces. The level of marker heterozygosity is in accordance with morphological variability observed in different horticultural types. Due to high cross-species transferability of EST-SSRs, even markers with very limited levels of heterozygosity that are not suitable for cultivar fingerprinting can be useful in the analysis of population structure and in genotyping *Lactuca* species. The EST-derived SSR markers identified in this work will be implemented in linkage map construction, cultivar genotyping, analysis of population structure in association studies, and comparative studies in *Lactuca* species. Moreover, markers polymorphic in 2 or more mapping populations will allow integrating the respective linkage maps.

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 (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 2008, we selected 62 PIs from an unreplicated experiment and 12 PIs from a single replicated experiment for further testing. In addition, seven advanced PIs were evaluated in their third replicated greenhouse experiment. Based on all three experiments, all seven had significantly lower disease incidence than Salinas and La Brillante. However, all of these PIs have had at least a few symptomatic plants, as well as non-symptomatic plants that are nonetheless colonized by *V. dahliae*. All 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 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 2008, requests were made for Verticillium wilt resistant breeding lines (RH05-0336, RH05-0339, and RH05-0340) and bacterial leaf spot resistant icebergs (RH07-0370M, RH07-0373M, RH07-0379M, RH07-0380M, RH07-0386M, RH07-0387M, and RH04-0157-3).

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 and resources for our trials.

E. Recent publications relevant to this project

Hayes, R. J, Ryder, E.J. and Wintermantel, W.M. 2008. Genetic variation for big-vein symptom expression and resistance to *Mirafiori lettuce big-vein virus* in *Lactuca virosa* L., a wild relative of cultivated lettuce. Euphytica 164:493-500.

Hayes, R. J., Ryder, E. J., and Bull, C.T. 2008. Notice of release of six F6:8 and one F4:5 iceberg breeding lines of lettuce germplasm with resistance to bacterial leaf spot caused by *Xanthomonas campestris pv. vitians*. (Germplasm Release).

Simko, I. 2009. Development of EST-SSR Markers for the Study of Population Structure in Lettuce (*Lactuca sativa* L.). Journal of Heredity 100: 256–262.

F. Appendix of Tables and Figures

Table 1. Crosses of new, potential sources of resistance to Fusarium wilt with susceptible and resistant cultivars.

Potential	Susceptible			Resistant	
source	Autumn Gold	Empire	Vanguard	Costa Rica No. 4	Salinas
917-0857	+	+	+	+	
Slugger	+	+	+	+	

SAWA UP	+	+		+	+
King Louie	+	+	+	+	+
Fresh Heart	+	+	+	+	+
Conquistador				+	
BOS 9021	+	+		+	
Apollo		+	+	+	+

+ indicates successful cross

Table 2. Corky root severity means and head characteristics of butterhead breeding lines and cultivars evaluated at the Spence Farm in Salinas, Calif. in summer 2008.

Genotype	Corky root[z]	Head Wt. (g)	Core length (cm)	Tipburn leaves[y]
Salinas	7.9 A	830.0 A	5.1 B	0.0 C
Cobham Green	7.9 A	207.5 E	2.8 C	1.5 ABC
Dark Green Boston	7.8 A	280.5 DE	4.8 B	2.3 A
Margarita	6.8 B	209.5 E	2.5 C	0.1 C
MU07-950-1	6.1 BC	562.5 B	2.6 C	0.1 C
Glacier	5.9 C	796.5 A	5.1 B	0.6 BC
MU07-902-1	5.9 C	463.8 C	7.7 A	0.0 C
MU07-953-1	5.9 C	294.8 DE	4.5 B	1.9 AB
MU07-955-1	5.9 C	413.3 C	6.6 A	1.9 AB
MU07-954-1	5.5 CD	311.0 D	4.4 B	1.0 ABC
MU06-815-1	5.0 D	420.5 C	7.4 A	0.9 ABC

[z] Means in the same column followed by different letters are sig. different 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 leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2008.

Genotype	Corky root[z]	Downy mildew	Plant Wt. (g)	Core length (cm)	Tipburn leaves[y]
Big Red	7.9 A	3.8 A	806.8 ABC	13.2 A	4.0 A
Prizehead	7.9 A	3.8 A	690.5 CD	5.9 DEFGH	0.0 C
Lolla Rossa	7.8 A	3.0 B	217.5 I	7.2 CDEFG	0.0 C
Redina	6.9 B	1.3 DE	357.0 GHI	10.6 B	0.0 C

Merlot	6.7 B	1.1 DE	326.8 HI	8.8 BC	0.0 C
MU07-787-1	6.0 C	2.0 C	690.0 CD	6.1 DEFGH	0.0 C
Glacier	5.9 C	4.0 A	796.5 ABC	5.1 GH	0.6 C
MU06-829-1	5.9 C	---	930.8 A	8.7 BC	1.0 BC
MU06-811-2	5.8 C	---	535.0 EF	5.4 FGH	0.0 C
MU07-553-2	5.6 C	1.1 DE	509.0 EF	4.5 H	0.0 C
MU07-553-1	5.6 C	1.0 E	490.0 EFG	4.2 H	0.0 C
MU06-828-1	5.5 C	---	859.8 AB	8.3 BCD	2.4 B
MU06-839-1	5.5 C	2.1 C	686.8 CD	5.4 FGH	0.0 C
MU07-553-3	5.5 C	1.4 D	584.3 DE	5.7 FGH	0.0 C
MU07-788	5.5 C	---	696.0 CD	5.8 EFGH	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 4. Mean values of corky root severity and head characteristics of green leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2008.

Genotype	Corky root[z]	Plant Wt. (g)	Core length (cm)	Tipburn leaves[y]
Grand Rapids	8.0 A	421.5 D	5.0 B	0.0 A
Shining Star	8.0 A	690.8 C	7.6 A	0.0 A
Waldmann's Green	8.0 A	526.3 D	9.1 A	0.0 A
Two Star	7.9 A	736.3 BC	4.8 B	0.0 A
Glacier	5.9 B	796.5 ABC	5.1 B	0.6 A
MU06-831-1	5.6 B	924.5 A	8.2 A	0.4 A
MU06-833-1	5.4 B	851.5 AB	7.4 A	0.0 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 5. Mean values of leafminer sting density and head characteristics of green leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2008.

Genotype[z]	Stings[y]/20 cm^2	Plant Wt. (g)	Core length (cm)
Two Star	162.6 A	736.3 B	4.8 D
Waldman's Green	131.5 B	526.3 CD	9.1 A
Shining Star	109.0 C	690.8 B	7.6 ABC
07-817-1 (*cor*)	46.6 D	583.8 C	4.8 D
05-917-2	40.9 DE	445.5 D	5.3 CD

06-844-1 (*cor*)	30.6 DE	893.5 A	7.9 ABC
06-841-1 (*cor*)	23.3 E	693.5 B	5.5 BCD

[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 6. Mean values of leafminer sting density and head characteristics of red leaf lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2008.

Genotype	Stings[z]/20 cm^2	Plant Wt. (g)	Core length (cm)
Prizehead	203.9 A	690.5 B	5.9 C
Big Red	170.4 B	806.8 A	13.2 A
Lolla Rossa	96.0 C	217.5 C	7.2 BC
07-838	46.8 D	667.8 B	8.2 BC
07-939-1	43.6 D	599.8 B	9.4 B

[z] Means in the same column followed by different letters are significantly different at $P < 0.0$

Table 7. Mean values of leafminer sting density and head characteristics of romaine lettuce breeding lines and cultivars evaluated in a trial at the Spence Farm in Salinas, Calif. in summer 2008.

Genotype	Stings[z]/20 cm^2	Plant Wt. (g)	Core length (cm)	Tipburn leaves
Green Forest	301.3 A	1,015.0 A	7.7 A	0.0 C
Darkland	267.9 AB	1,112.5 A	7.3 A	2.0 BC
Heart's Delight	236.0 B	1,212.5 A	9.5 A	2.5 BC
Clemente	229.5 B	1,173.5 A	9.2 A	3.4 B
06-896-1	94.3 C	1,132.0 A	9.5 A	6.8 A

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

Table 8. 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 2008.

Genotype	Stings[z]/20 cm^2	Head Wt. (g)	Core length (cm)	Tipburn leaves
Bronco	250.8 A	730.8 BC	4.7 CD	0.0 B
Premier	236.2 A	660.8 C	3.4 D	0.0 B
Sniper	234.4 A	939.5 A	7.4 AB	0.1 B
06-859-2	84.1 B	816.0 B	6.4 BC	0.3 B
07-810-1	72.6 B	685.0 C	9.5 A	1.0 A

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

0.05.

Table 9. Resistance to leafminer stings in the F_2 generation and chi square (χ^2) analysis of F_3 populations for their fit to a monogenic dominant inheritance model for resistance in a cross (PI 509525 x Da Ye Wo Sun) evaluated in Salinas, California in 2008.

F3 family	Reaction of F2 plant	Resistant (R) plants[z]	Susceptible (S) plants	*P* 3:1
07-1019-18	S	0	30	
07-1030-18	S	0	122	
07-1019-14	R	31	0	
07-1020-19	R	13	0	
07-1025-19	R	67 (65)	20 (22)	0.50-0.75
07-1033-5	R	94 (91)	28 (31)	0.50-0.75

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[z] Numbers in parentheses are expected values for a 3:1 segregation.

Table 10. Resistance to downy mildew in the F_2 generation and chi square (χ^2) analysis of F_3 populations for their fit to a monogenic dominant inheritance model for resistance in a cross (PI 509525 x Da Ye Wo Sun) evaluated in Salinas, California in 2008.

F3 family	Reaction of F2 plant	Resistant (R) plants[z]	Susceptible (S) plants	*P* 3:1
07-1019-18	S	0	29	
07-1030-18	S	0	101	
07-1019-14	R	31	0	
07-1025-19	R	83	0	
07-1033-5	R	114	0	
07-1020-19	R	11 (10)	3 (4)	0.50-0.75

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[z] Numbers in parentheses are expected values for a 3:1 segregation.

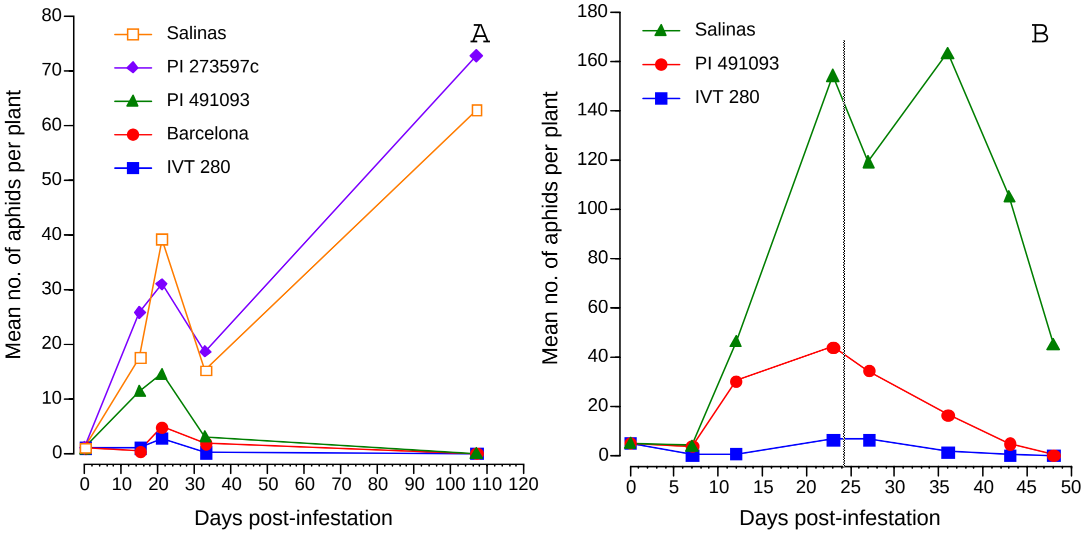

Fig. 1. Mean number of lettuce aphids per plant, A) Developed from a single 24 to 48-hr old nymph placed on each plant; greenhouse. B) Developed from five 24 to 48-hr old nymphs placed on each plant; entries were placed into separate cages following the counts 23 dpi; greenhouse.

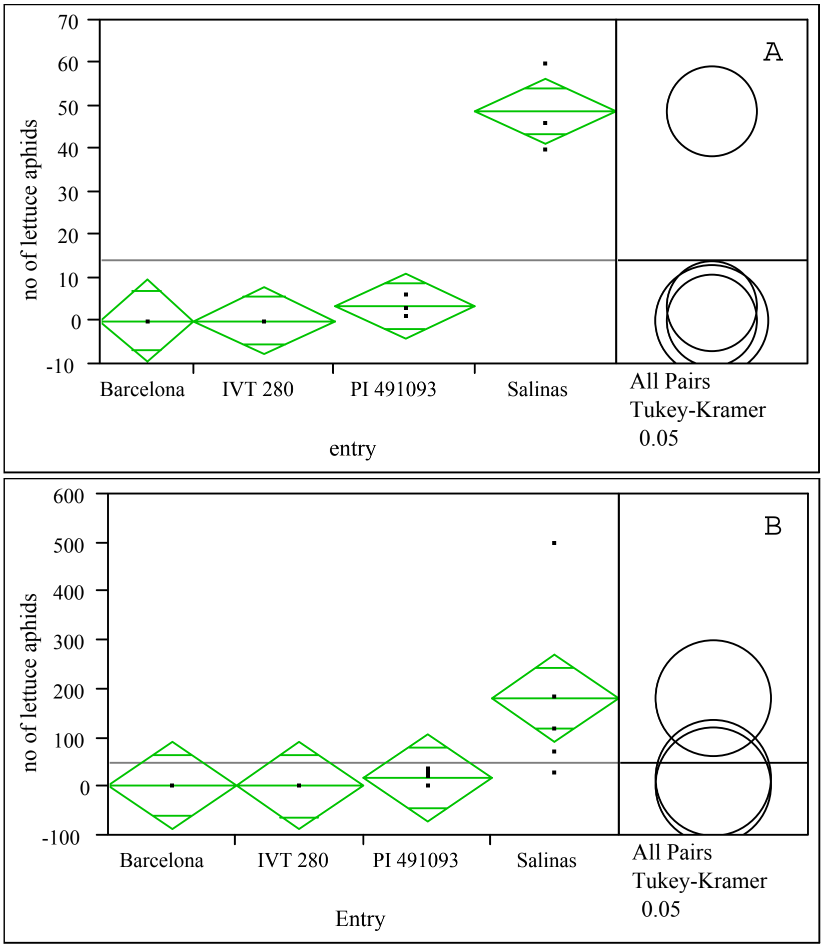

Fig. 3. A) Mean numbers of lettuce aphids per plant 31 dpi that developed from five, 24 to 48-hr old nymphs placed on each plant; field, plants in individual cages. B) Mean numbers of lettuce aphids per plant from natural infestation in an open field; counted near maturity of 'Barcelona' and 'Salinas'.

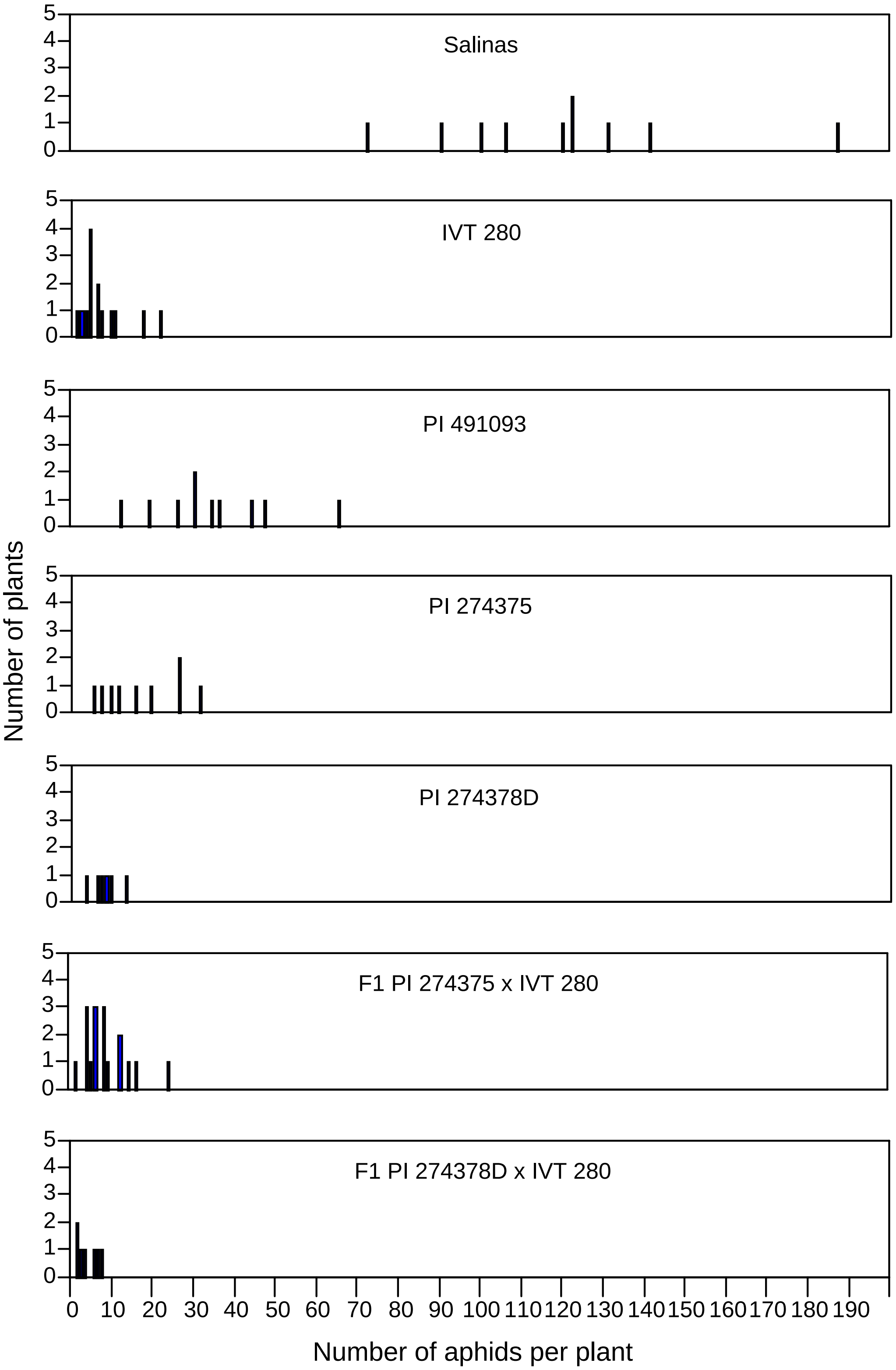

Fig. 3. Frequency distributions of numbers of plants with different numbers of aphids 27 dpi; greenhouse