

CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

April 1, 2008 to March 31, 2009

BREEDING CRISPHEAD LETTUCE

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SUMMARY

The program continues to emphasize the identification and incorporation of genes for disease resistance, particularly to downy mildew, *Verticillium* and *Fusarium* wilts, corky root, lettuce mosaic virus, and anthracnose, into crisphead horticultural types suitable for California. Resistance for downy mildew is being introduced from several new sources and combined with resistance to lettuce mosaic virus and corky root. We have continued to monitor variation in the ability of the downy mildew pathogen to overcome resistance genes. There have been further increases in variation in the pathogen. Of the known resistance genes, only *Dm17* remains effective against all California isolates. Utilization of multiple new sources will minimize the chances that changes in the pathogen will render all cultivars susceptible simultaneously. Advanced lines are trialed in Salinas. We have initiated a program for thermo-tolerance during seed germination for desert types.

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OBJECTIVES:

- 1) To identify new genes for disease resistance in wild germplasm and incorporate multiple genes from diverse sources into advanced breeding lines to achieve durable resistance.
- 2) To monitor variation in pathogen populations, particularly downy mildew, to facilitate the deployment of effective resistance genes.
- 3) To determine the genetic basis of agriculturally important traits, particularly disease resistance.
- 4) To release advanced crisphead breeding lines which have resistance to multiple diseases, superior appearance and quality, high yielding ability, uniform maturity, and are slow bolting.

PROCEDURES AND RESULTS:

Development of Disease Resistant Lines

Downy mildew: We are continuing to develop crisphead coastal lettuce lines with resistance genes from diverse sources to provide protection against downy mildew in California. We are now focused on generating advanced breeding lines with new resistance genes (Table 1). Crosses have been and are being made to combine lettuce downy mildew (LDM) resistance with genes for resistance to other diseases. These resistant accessions are different from those being used as donors for resistance in the leafy program. This will diversify the selection pressure on the pathogen. The use of multiple sources of resistance will tend to increase the longevity of each resistance gene and decrease the chances that a single change in the pathogen will render multiple lettuce types susceptible.

New germplasm screens to identify additional sources of LDM resistance were initiated in 2007 and continued through 2008. Ninety-five accessions of *Lactuca serriola* from Ales Lebeda (Palacký University, Olomouc, Czech Republic) were screened for resistance to a range of California isolates representing the most virulent isolates currently identified. Fifteen accessions were resistant to all isolates tested. Five of these were crossed to cv. Salinas; other accessions will be used as donors in the leafy program. Sixty-six accessions of *L. saligna*, also from Ales Lebeda, were screened against the most virulent Californian isolates and 30 accessions were identified as resistant to all 30 isolates. Five of these are being used as resistance donors in the crisphead program and others will be used in the leafy program. Backcrossing programs to introgress the next generation of resistance to LDM have been initiated (Table 1). Among these new sources, UC04US2509 has already been characterized genetically as containing a new LDM resistance gene (*Dm45*).

Table 1: Status of new sources of resistance to downy mildew

Source	<i>Lactuca</i>	Current status	Resistance genes
PI491226	<i>sativa</i>	Released 2008	<i>Dm41</i> *
PI491108	<i>serriola</i>	Released 2008	<i>Dm42</i> *
PI491206	<i>saligna</i>	Released 2008	<i>Dm43</i> *
PI491208	<i>saligna</i>	Released 2008	<i>Dm44</i> *
UC94Isr1	<i>saligna</i>	To be released 2009	
CGN9311	<i>saligna</i>	To be released 2009	
CGN5318	<i>saligna</i>	To be released 2009	
CGN5282	<i>saligna</i>	To be released 2009	
CGN5147	<i>saligna</i>	To be released 2009	
UC04US2509	<i>saligna</i>	BC ₆	<i>Dm45</i>
UC04US2507	<i>virosa</i>	BC ₅	
5 accessions	<i>serriola</i>	BC ₂	
5 accessions	<i>saligna</i>	F ₁	

*: Provisional resistance gene designation.

Downy mildew virulence surveys. In order to ensure that we are breeding for resistance against virulence phenotypes of the pathogen currently present in California, we have continued to sample the downy mildew pathogen on an opportunistic basis with the collaboration of growers, the seed industry and extension personnel, particularly Steve Koike. Over the past year, 33 isolates were characterized for virulence phenotype, fungicide sensitivity and mating type (Table 2). This opportunistic sampling provides an indication of the diversity in the field but does not provide a quantitative measure of LDM variation. CAVIII was the predominant pathotype identified in 66% of the samples analyzed. About 10% of the samples were characterized as CAVII and 25% were novel types. California pathotypes V and VI were not found among these samples (Figure 1).

All isolates sampled in 2008 expressed *Avr17* (were avirulent of *Dm17*). *Avr36*, *Avr37* and *Avr38* were detected in frequencies higher than 60%. The frequency of *Avr18* dropped considerably in 2008 to lower than 5%. *Avr4* was detected at a frequency of 15%. Among the novel isolates, *Avr2*, *Avr3* and *Avr6* were detected at low frequencies (Figure 2). This is interesting because until recently avirulence to *Dm2* or *Dm3* was very rarely detected in California. The presence of *Avr2* and *Avr3* may indicate that the recently identified novel isolates have originated from different source(s) than isolates characterized previously.

In order to allow comparison of California isolates with those described using the European system (K. van Ettehoven and A. van der Arend, 1999. *Eucarpia Leafy Vegetables*, A. Lebeda and E. Kristkova, eds. pp171-175), we have included the sextet code to describe the phenotype of isolates in Table 2. Sextet code values were determined using the same differential series of resistant cultivars as used in the European classification. However, a variety carrying a new resistance will not necessarily have the same reaction to isolates of the same sextet code from the different continents. Therefore, the pathotype and race designations for isolates from California and Europe have been maintained and should be considered together.

All isolates characterized in 2008 were B₂ mating type and sensitive to the fungicides metalaxyl and Aliette (Table2). Therefore both of these fungicides currently have the potential to provide control in California. However, the pathogen should be continuously monitored for the reappearance of insensitivity to these fungicides to prevent ineffective applications of fungicide.

Figure 1: Frequency of Downy Mildew Pathotypes in California, 2003 – 2008.

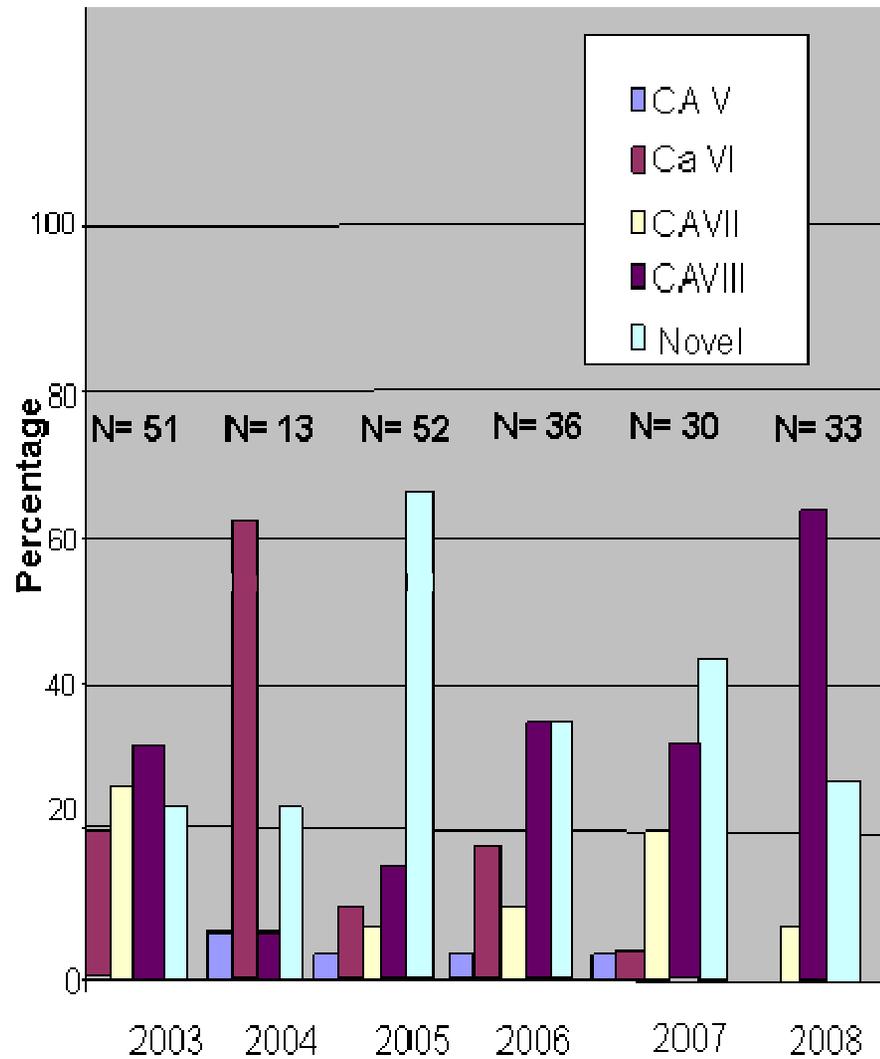


Figure 2: Frequency of Avirulence Genes Detected in 2008

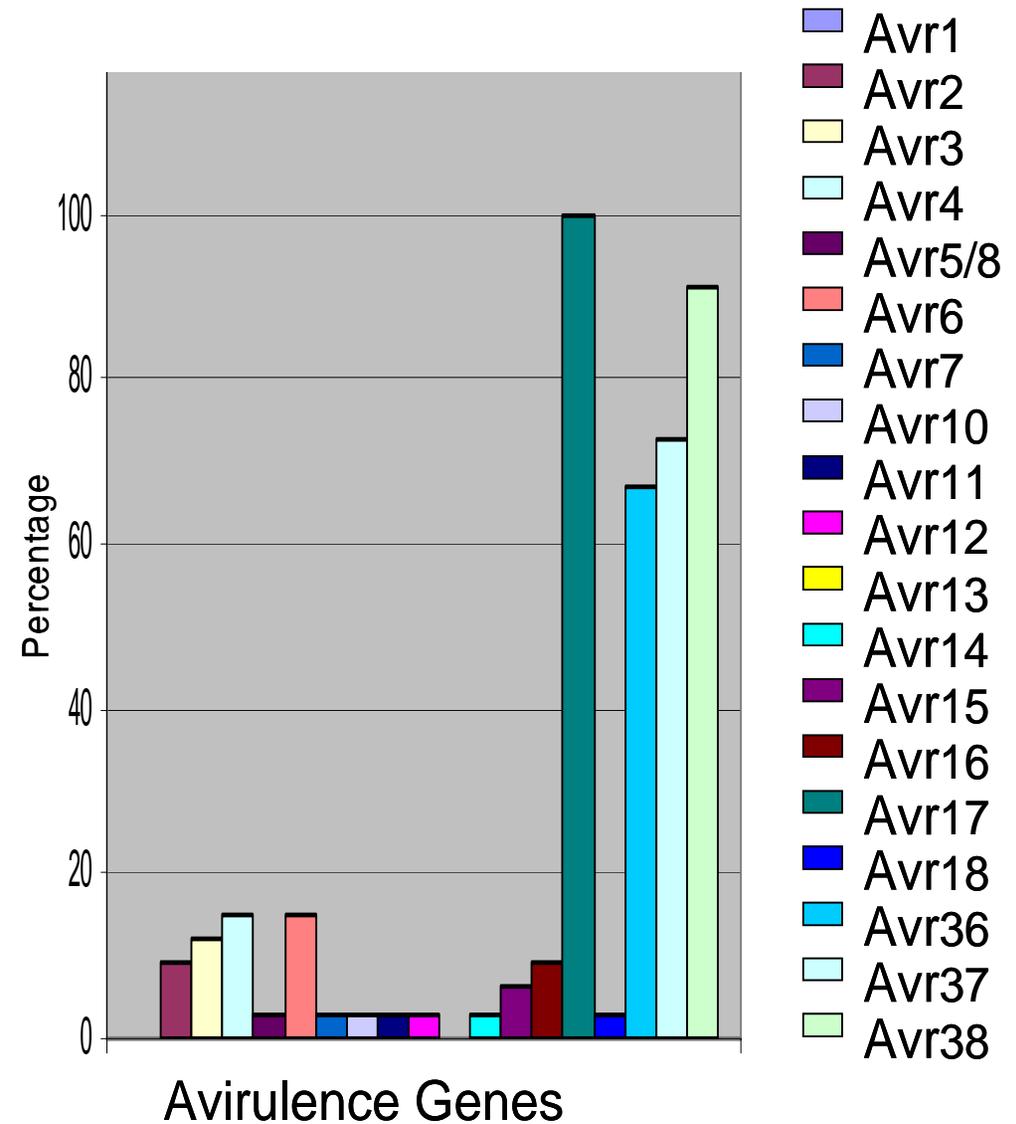


Table 2. Virulence phenotypes of isolates of *B. lactucae* characterized in 2008.

			Cobham Green	Lednický	UC DME	Dandle	RAT57D	Valmaine	Sabine	LSE 57/15	UC DM10	Capitan	Hilde II	Pennlake	UC DM4	PIVT 1309	LSE 18	LS-102	Colorado	Minja	Discovery	Argedes	Mariska	El Dorado	R 32	Amplus	Pathotype													
			DMR Genes	0	1	2	3	4	5	6	7	10	11	12	13	14	15	16	17	18	36	37	38	10m	10ed	18/32	40													
2008			Sextet location	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23														
Isolate ID	Location	Col.	Sextet value	1	2	4	8	16	32	1	2	4	8	16	32	1	2	4	8	16	32	1	2	4	8	16		Sextet 1	Sextet 2	Sextet 3	Sextet 4									
																															Fully avirulent				Mating			Fung Test		
																																			Alliete			Ridomil		
A0801160	Somerton, AZ	M. Matheron		+	+	-	-	+	+	-	-	+	-	-	+	-	-	-	+	-	-	-	-	+	+	+	-	Novel	25	18	8	0	2,3,6,11,12,14,15,16,17,36,37,38				B2	SEN	SEN	
C0801161	Brawley, CA	S. Koike		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-	CA VIII	63	63	11	0	17,36,37,38,40				B2	SEN	SEN		
C0801162	Brawley, CA	S. Koike		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-	CA VIII	63	63	11	0	17,36,37,38,40				B2	SEN	SEN		
C0801163	Brawley, CA	S. Koike		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-	CA VIII	63	63	11	0	17,36,37,38				B2	SEN	SEN		
C0801164	Brawley, CA	S. Koike		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-	CA VIII	63	63	11	0	17,36,37,38,40				B2	SEN	SEN		
C0801165	Brawley, CA	S. Koike		+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-	Novel	57	63	11	0	2,3,17,37,38				B2	SEN	SEN		
C0801166	Davis, CA	O. Ochoa		+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-	+	+	-	-	+	Novel	47	61	19	1	5/8,10,17,37,18c,18m,18ed,18/32				B2	SEN	SEN			
C0801167	?, CA	S. Koike		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	CA VIII	63	63	59	0	17,38,40				B2	SEN	SEN			
C0801168	Ardo, CA	J. Heinzberger		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	CA VIII	63	63	59	1	17				B2	SEN	SEN			
C0801169	Mosslanding, CA	J. Heinzberger		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	CA VIII	63	63	11	0	17,36,37,38,40				B2	SEN	SEN			
C0801170	Spence(USDA), CA	I. Simko		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	CA VIII	63	63	11	0	17,36,37,38,40				B2	SEN	SEN			
C0801171	Spence(USDA), CA	I. Simko		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	CA VIII	63	63	11	0	17,36,37,38,40				B2	SEN	SEN			
C0801172	Spence(USDA), CA	I. Sirrko		+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	Novel	23	63	43	1	4,6,17,36				B2	SEN	SEN			
C0801173	Salinas, CA	O. Ochoa		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	Novel	63	63	9	0	16,17,36,37,38,40				B2	SEN	SEN			
C0801174	Salinas, CA	O. Ochoa		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	CA VIII	63	63	11	0	17,36,37,38,40				B2	SEN	SEN			
C0801175	Salinas, CA	O. Ochoa		+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+	+	Novel	31	63	10	0	6,15,17,36,37,38				B2	SEN	SEN			
C0801176	Salinas, CA	O. Ochoa		+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	CA VII	55	63	11	0	4,17,36,37,38				B2	SEN	SEN			
C0801177	Salinas, CA	O. Ochoa		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	CA VIII	63	63	27	0	17,36,37,38,40				B2	SEN	SEN			
C0801178	Salinas, CA	O. Ochoa		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	CA VIII	63	63	27	0	17,36,37,38,40				B2	SEN	SEN			
C0801181	Salinas, CA	oo/MT		+	+	+	-	+	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	Novel	27	63	43	0	3,6,17,36,38,40				B2	SEN	SEN			
C0801182	Salinas, CA	oo/MT		+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	Novel	57	63	11	0	2,3,17,36,37,38				B2	SEN	SEN			
C0801183	Salinas, CA	oo/MT		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	CA VIII	63	63	11	0	17,18M,36,37,38				B2	SEN	SEN			
C0801184	Salinas, CA	oo/MT		+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	CA VII	55	63	27	0	4,17,18M,36,37,38				B2	SEN	SEN			
C0801185	Salinas, CA	oo/MT		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	CA VIII	63	63	59	0	17,38				B2	SEN	SEN			
C0801186	Salinas, CA	oo/MT		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	CA VIII	63	63	59	0	17,38				B2	SEN	SEN			
C0801187	Salinas, CA	oo/MT		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	CA VIII	63	63	11	0	17,36,37,38				B2	SEN	SEN			
C0801189	Salinas, CA	Beguan		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	CA VIII	63	63	59	0	17,38				B2	SEN	SEN			
C0801190	Salinas, CA	Beguan		+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	CA VII	55	63	11	0	17,36,37,38				B2	SEN	SEN			
C0801191	Salinas, CA	Beguan		+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+	Novel	23	63	9	0	4,6,16,17,36,37,38				B2	SEN	SEN			
C0801192	Salinas, CA	Beguan		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	CA VIII	63	63	59	0	17,38,18m				B2	SEN	SEN			
C0801193	?, CA	S. Koike		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	CA VIII	63	63	11	0	17,36,37,38				B2	SEN	SEN			
C0801194	?, CA	S. Koike		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	CA VIII	63	63	11	0	17,36,37,38				B2	SEN	SEN			
C0801195	?, CA	Shamrock		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	CA VIII	63	63	59	0	17,38				B2	SEN	SEN			

^a: Sextet location 20 to 23 are not considered within the sextet codes.

Verticillium Wilt: Resistance to Verticillium wilt is now a top priority for our program. We have developed an efficient, reliable, and contained method for screening for the reaction of lettuce to *Verticillium dahliae* in the greenhouse. We are currently utilizing microplots within the greenhouse and have restricted access to them to minimize the opportunity for spread of the pathogen (Figure 3). We are screening for resistance to *V. dahliae* strain VdLs17 (race 2) provided by Dr. Krishna Subbarao. We include Salinas as the susceptible control genotype with La Brillante representing a genotype that has shown less disease in the field.

Over the past three years, 333 accessions have been screened against race 2 including 21 *L. sativa*, 114 *L. saligna*, 183 *L. serriola* and 15 *L. virosa* accessions. None of the accessions showed full resistance. A few accessions showed less severe symptoms and they have been inter-crossed to analyze the genetics of this phenotype as well to quantify the pathogen in progeny with various levels of symptom severity. New germplasm is being gathered from different sources (USDA, CGN, & other collections) to continue the search for resistance against race 2.

Figure 3. Microplots in containment tanks within the greenhouse used to screen lettuce for reaction to Verticillium wilt.



PCR analysis to quantify the amount of Verticillium in plants exhibiting different amounts of disease symptoms showed that while some asymptomatic plants had less Verticillium than plants with severe symptoms, other asymptomatic plants had high levels

of the pathogen (see Leafy Report). Therefore symptoms alone are not a reliable indicator of resistance or tolerance to the pathogen.

We have inter-crossed plants that exhibited reduced symptoms in the germplasm screens. Their F₂ progeny will be screened to determine whether there is transgressive segregation for resistance and whether we can generate lines with high levels of resistance using marker-assisted selection.

We also screened the parental lines used in our mapping population as well as the donors for our breeding populations for resistance to isolates Vdls14 (race 1) and Vdls17 (race 2). All parental lines were susceptible to both races and therefore none of our existing mapping and breeding populations are informative for resistance to *Verticillium*. In collaboration with Dr. Ryan Hayes (USDA), we analyzed the RIL (Recombinant Inbred Line) population from a La Brillante x Salinas 88 cross in order to map resistance to race 1 in La Brillante. We also are incorporating this resistance into our breeding program in order to provide resistance against race 1 until resistance is found against race 2. Additional details of breeding for resistance to *Verticillium* are included in the Leafy Breeding Report.

Multiple Disease Resistances

Crosses between advanced breeding lines, field selections, and released lines have been made to generate lines with multiple disease resistances. Screening for multiple diseases, including lettuce downy mildew, corky root, anthracnose and lettuce mosaic virus, continues.

Thermotolerance

We are collaborating with Kent Bradford (UC Davis) to incorporate thermotolerance for seed germination into crisphead breeding lines for the dessert. We are using molecular markers linked to a major quantitative trait locus that confers the ability to germinate at high temperatures to introgress an allele from *L. serriola* acc. UC96US23.

Trials of Breeding Lines

The program continues the strategy of crosses being made and early generations being grown at Davis with later generations being trialed and selections made from those field trails in collaboration with Richard Smith. Backcross or modified single-seed descent strategies are being employed for most early generations. We continue to select for good color, slow bolting, and yield as well as disease resistance in cv. Salinas plant types. Two trials were planted in 2008 for the crisphead program and five for the leafy program (Table 3).

Table 3. Trials planted in 2008.

Lettuce trial	Grower	Location	Planting date	Plant material
Winter Leaf	Boutonnet Farms	Hunter Lane Ranch, Lot 17; Hunter Lane, west of Salinas	03/03/08	9 lines
Winter Head	Boutonnet Farms	Jacop Ranch, Lot 3: Davis Road southwest of Salinas	03/03/08	12 lines
Summer Leaf	Royal Packing	Hooker Ranch Block 9A	06/19/08	20 lines
Summer Head	Royal Packing	Cooper Ranch Block 15N	06/02/08	15 lines
Fall Leaf	Boutonnet Farms	Hurley Ranch	08/08/08	25 lines
Fall Baby Leaf	Marvin Borzini with Metz Fresh	San Lucas	08/22/08	3 releases
Fall Leaf/Head	USDA, Salinas	Field Station	08/06/08	7 releases + 40 lines

Releases of Lines Resistant to Downy Mildew

We released four advanced crisphead breeding lines in March, 2008. Sixteen different seed companies and research groups have requested these releases thus far. Interested groups should request the releases of head and leafy types by contacting us by mail or e-mail.

Supply of Isolates

We have continued to supply California isolates of downy mildew and corky root to breeding companies and other research groups. We have trained personnel from the seed industry and others to handle lettuce downy mildew, corky root, anthracnose and other diseases.