CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

April 1, 2009 to March 31, 2010

BREEDING CRISPHEAD LETTUCE

Richard W. Michelmore
Oswaldo E. Ochoa
The Genome Center and
The Department of Plant Sciences
University of California, Davis
rwmichelmore@ucdavis.edu
oeochoa@ucdavis.edu

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 genes for resistance to downy mildew, only *Dm17* remains effective against all of the California isolates tested. 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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PROJECT TITLE: **BREEDING CRISPHEAD LETTUCE**

PRINCIPAL INVESTIGATORS: **Richard W. Michelmore**
Oswaldo E. Ochoa
The Genome Center and
The Department of Plant Sciences
University of California, Davis
rwmichelmore@ucdavis.edu
oeochoa@ucdavis.edu

COOPERATING PERSONNEL: **Maria José Truco**
mjtruco@ucdavis.edu
Ryan Hayes
UDSA-ARS, Salinas
Krishna Subbarao
The Department of Plant Pathology
University of California, Davis
Richard Smith
UC-Cooperative Extension, Monterey County

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 2009. 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*). Mapping and characterization of the new sources are in progress.

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, 51 isolates were characterized for virulence phenotype and a subset of them were characterized for 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. CAVII was the predominant pathotype, identified in 49 % of the samples analyzed in 2009. About 8 % of the samples were characterized as CAVIII and 35 % were novel types. California pathotypes V and VI were not detected over the past year amongst the samples analyzed (Figure 1).

All isolates sampled in 2009 expressed *Avr17* (were avirulent to *Dm17*). *Avr36*, *Avr37* and *Avr38* were detected in frequencies higher than 80 %. The frequency of *Avr18* dropped to 0 %. *Avr4* was detected at a frequency of 51 %. Among the novel isolates, *Avr2*, *Avr3* and *Avr6* were detected at 20 %, 28 % and 18 % frequencies respectively (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 Ettekoven 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.

Few of the isolates were characterized in 2009 for mating type and sensitivity to the fungicides metalaxyl and Aliette (Table 2). All those tested were B_2 and sensitive to both fungicides.

Table 1: Status of breeding with new sources of resistance to downy mildew.

Source		Donor Species	Status	R gene *
PI491226	USDA	*L. serriola*	2007 Release UC07105	41
PI491108	USDA	*L. serriola*	2007 Release UC07106	42
PI491206	USDA	*L. saligna*	2007 Release UC07107	46
PI491208	USDA	*L. saligna*	2007 Release UC07108	47
CGN9311	CGN	*L. saligna*	To be released 2010	
CGN5318	CGN	*L. saligna*	To be released 2010	
CGN5282	CGN	*L. saligna*	To be released 2010	
CGN5147	CGN	*L. saligna*	To be released 2010	
UC94lsr1	UC	*L. saligna*	To be released 2010	
UC04US2509	UC	*L. saligna*	BC6 Trials 2010	45
UC04UK2507	UC	*L. virosa*	BC6	
UC07CS100	LEB-CS	*L. serriola*	BC3	48
UC07CS101	LEB-CS	*L. serriola*	BC3	49
UC07CS102	LEB-CS	*L. serriola*	BC3	
UC07CS103	LEB-CS	*L. serriola*	BC3	
UC07CS104	LEB-CS	*L. serriola*	BC2	
UC07CS105	LEB-CS	*L. saligna*	BC2	
UC07CS106	LEB-CS	*L. saligna*	BC2	
UC07CS107	LEB-CS	*L. saligna*	BC2	
UC07CS108	LEB-CS	*L. saligna*	BC1	
UC07CS109	LEB-CS	*L. saligna*	BC1	

*: Provisional resistance gene designation.

Figure 1: Frequency of Downy Mildew Pathotypes in California, 2004 – 2009.

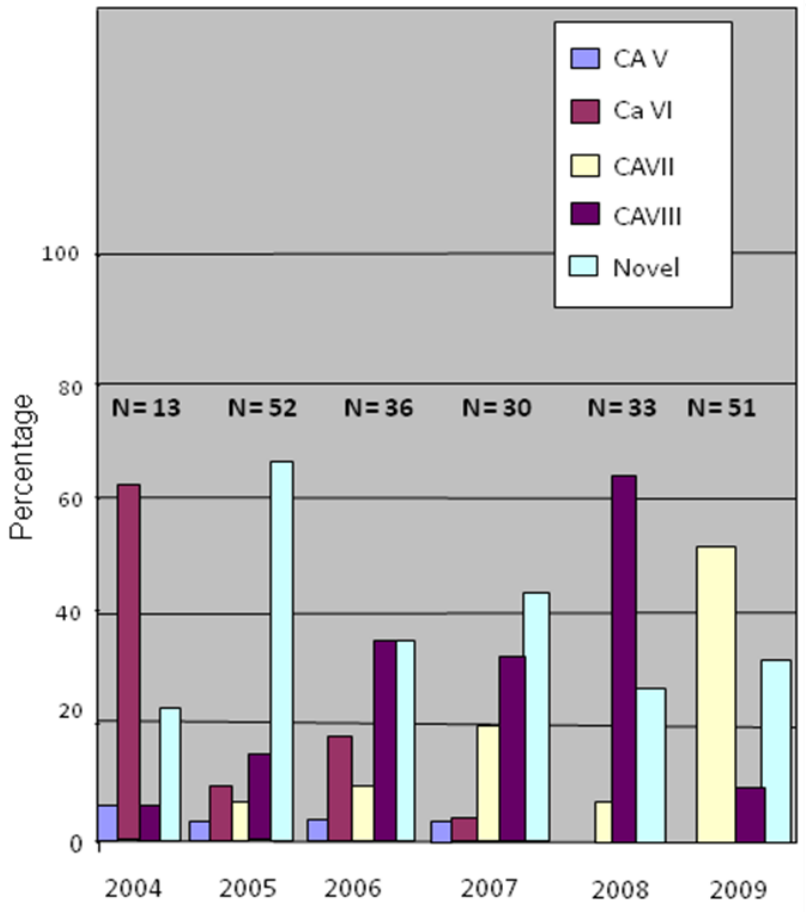

Figure 2: Frequency of Avirulence to *Dm* Genes. Effective *Dm* genes and R-factors are circled

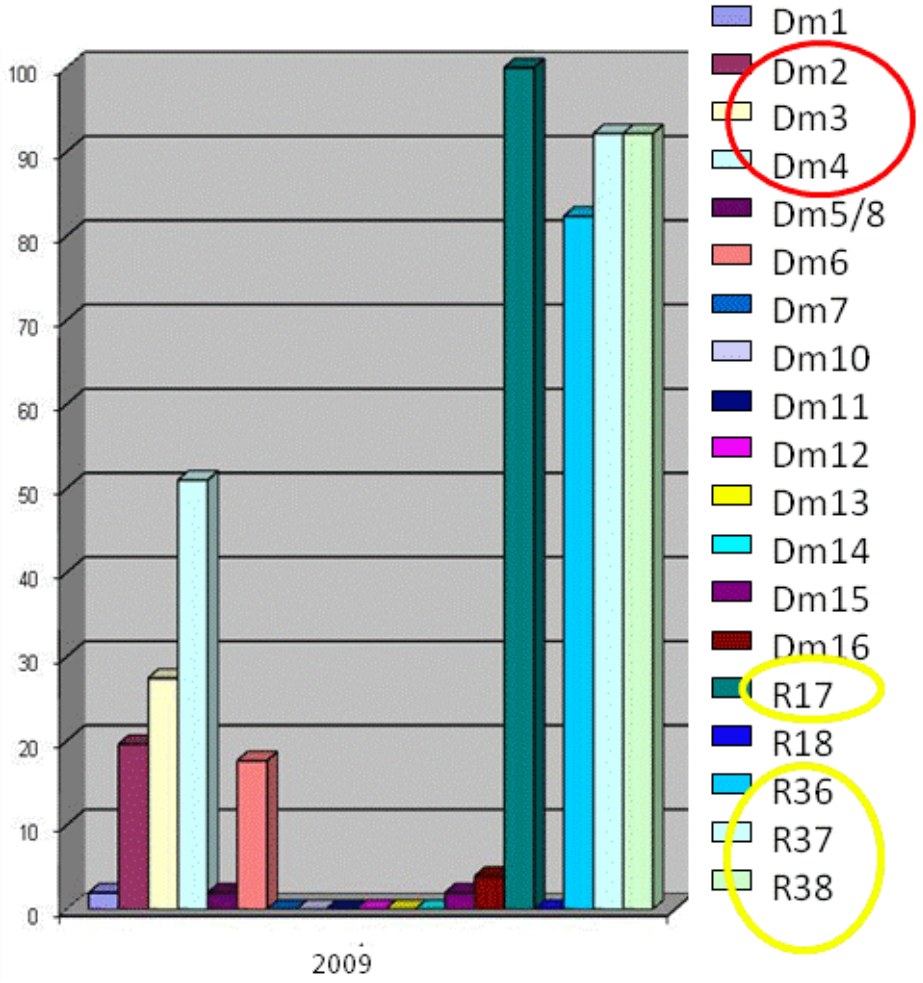

Table 2: Virulence phenotype of isolates of *B. lactucae* characterized in 2009 from opportunistic sampling.

		Cobham Green	Ledmicky	UC DM2	Dandie	R4T57D	Valmaine	Sabine	LSE 57/15	UC DM10	Capitan	Hilde II	Penniake	UC DM14	PiVT 1309	LSE /18	LS-102	Colorado	Ninja	Discovery	Argeles	Mariska	El Dorado	R32	Amplus	Sextet code	Sextetblok 1	Sextetblok 2	Sextetblok 3	Sextetblok 4				
	DM/R Genes	0	1	2	3	4	5/8	6	7	10	11	12	13	14	15	16	17	18	36	37	38	18m	18ed	18/32	40									
	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								Fung Test	
Isolate ID	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						Fully avirulent	Pathotype	Mating	Alliete	Ridomil
2009																																		
C09D1196		+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-	+		46	63	27	0	1,5/8,17,37,38	Novel	B2	Sen	Sen
C09O1197		+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	-		57	63	11	1	2,3,17,36,37	Novel	B2	Sen	Sen
C09O1198		+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	-		61	63	11	1	2,17,36,37	Novel	B2	Sen	Sen
C09D1199		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	+	-		55	63	11	0	4,17,36,37,38,	CA-VII	B2	Sen	Sen
C09D1201		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		55	63	11	0	4,17,36,37,38	CA-VII	B2	Sen	Sen
C09D1202		+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		23	63	11	0	4,6,17,36,37,38	Novel	B2	Sen	Sen
C09D1203		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		55	63	11	0	4,17,36,37,38,	CA-VII	B2	Sen	Sen
C09D1204		+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	+	-		23	63	11	0	4,6,17,36,37,38	Novel	B2	Sen	Sen
C09D1205		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	+	-		55	63	11	0	4,17,36,37,38	CA-VII	B2	Sen	Sen
C09D1206		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		55	63	11	0	4,17,36,37,38	CA-VII	B2	Sen	Sen
C09D1207		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		55	63	11	0	4,17,36,37,38	CA-VII	B2	Sen	Sen
C09D1208		+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		57	63	11	0	2,3,17,36,37,38	CA-VII	B2	Sen	Sen
C09D1210		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		63	63	11	0	17,36,37,38,	Ca-VIII	B2	Sen	Sen
C09D1211		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+		55	63	11	0	4,17,36,37,38	CA-VII	B2	Sen	Sen
C09D1212		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+		55	63	11	0	4,17,36,37,38	CA-VII	B2	Sen	Sen
C09D1216		+	+	+	+	-	+	+	+	+	+	+	nd	+	+	+	-	+	-	-	-	+	+	+	nd		55	47	11	0	4,17,36,37,38	CA-VII	B2	Sen	Sen
C09D1217		+	+	+	+	-	+	+	+	+	+	+	nd	+	+	+	-	+	-	-	-	+	+	+	nd		55	47	11	0	4,17,36,37,38	CA-VII	B2	Sen	Sen
C09D1218		+	+	+	+	-	+	+	+	+	+	+	nd	+	+	+	-	+	-	-	-	+	+	+	nd		55	47	11	0	4,17,36,37,38	CA-VII	B2	Sen	Sen
C09D1220		+	+	+	+	+	+	+	+	+	+	+	nd	+	+	-	-	+	-	-	-	+	+	+	nd		63	47	9	0	16,17,36,37,38	Novel	B2	Sen	Sen
C09D1223		+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	+	+	+	+		59	63	10	0	3,15,17,36,37,38	Novel	B2	Sen	Sen
C09D1225		+	+	+	+	-	+	+	+	+	+	+	nd	+	+	+	-	+	-	-	-	+	+	+	nd		55	47	11	0	4,17,36,37,38	CA-VII	nd	nd	nd
C09D1227		+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+	+	nd		59	63	9	0	3,16,17,36,37,38	Novel	nd	nd	nd
C09D1228		+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	nd		59	63	11	0	3,17,36,37,38	Novel	nd	nd	nd
C09D1229		+	+	+	+	-	+	+	+	+	+	+	+	+	+	nd	-	+	-	-	-	+	+	+	nd		55	63	9	0	4,17,36,37,38	CA-VII	nd	nd	nd
C09D1230		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	+	nd		55	63	11	0	4,17,36,37,38	CA-VII	nd	nd	nd
C09D1231		+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	nd		27	63	11	0	3,6,17,36,37,38	Novel	nd	nd	nd
C09D1232		+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	nd		59	63	11	0	3,17,36,37,38	Novel	nd	nd	nd
C09D1233		+	+	+	+	-	+	+	+	+	+	+	+	+	+	nd	-	+	-	-	-	+	+	+	-		55	63	9	0	4,17,36,37,38	CA-VII	nd	nd	nd
C09D1234		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		55	63	11	0	4,17,36,37,38	CA-VII	nd	nd	nd
C09D1235		+	+	+	+	-	+	+	+	+	+	+	+	+	nd	+	-	+	-	-	-	+	+	+	-		55	63	10	0	4,17,36,37,38	CA-VII	nd	nd	nd
C09O1236		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	+	+		55	63	11	0	4,17,36,37,38	CA-VII	nd	nd	nd
C09O1237		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	-	-		55	63	27	0	4,17,37,38	CA-VII	nd	nd	nd
C09D1238		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		55	63	11	0	4,17,36,37,38	CA-VII	nd	nd	nd
C09O1239		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		55	63	11	0	4,17,37,38	CA-VII	nd	nd	nd
C09D1240		+	+	+	+	-	+	-	+	+	+	+	+	+	+	nd	-	+	+	+	-	-	+	+	-		23	63	57	0	4,17,38	CA-VII	nd	nd	nd
C09D1241		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	-		55	63	27	0	4,17,37,38	CA-VII	nd	nd	nd
C09D1242		+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-	-		55	63	27	0	4,17,37,38	CA-VII	nd	nd	nd
C09D1243		+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		25	63	11	0	2,3,17,36,37,38	Novel	nd	nd	nd
C09D1245		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	+	+		63	63	27	0	17,37,38	CA VIII	nd	nd	nd
C09O1247		+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		25	63	11	0	2,3,6,17,36,37,38	Novel	nd	nd	nd
C09O1248		+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		57	63	11	0	2,3,17,36,37,38	Novel	nd	nd	nd
C09O1249		+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		25	63	11	0	2,3,6,17,36,37,38	Novel	nd	nd	nd
C09O1250		+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		25	63	11	0	2,3,6,17,36,37,38	Novel	nd	nd	nd
C09O1251		+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		57	63	11	0	2,3,17,36,37,38	Novel	nd	nd	nd
C09O1252		+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		25	63	11	0	2,3,6,17,36,37,38	Novel	nd	nd	nd
C09D1253		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+		63	63	59	1	17	CA VIII	nd	nd	nd
C09D1254		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+		63	63	11	1	17,36,37	CA VIII	nd	nd	nd
C09D1255		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+		63	63	59	0	17,38	CA VIII	nd	nd	nd
C09D1256		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+		63	63	27	0	17,37,38	CA VIII	nd	nd	nd
C09O1257		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	+	+	+		63	63	43	0	17,36,38	CA VIII	nd	nd	nd
C09O1258		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-		63	63	11	0	17,36,37,38	CA VIII	nd	nd	nd

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. In collaboration with Ryan Hayes, new germplasm is being gathered from several different sources 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 F_2 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 and donor lines tested were susceptible to both races and therefore none of our existing mapping and breeding populations are useful 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 and mapped a major QTL for resistance to race 1 originating from La Brillante. We 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 thermo-tolerance 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 2009 for the crisphead program and three for the leafy program (Table 3).

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.

Table 3. Field Trails Planted in 2009.

Lettuce trial	Grower	Location	Planting date	# lines
Spring Leaf		Molera Ranch	March 26, 2009	10 lines
Spring Head	Boutonnet Farms	Bogiatto Ranch	April 1,2009	10 lines
Summer Baby Lettuce-Leaf	American Farms	Wilson Ranch	June 4, 2009	6 lines
Fall Romaine	D'Arrigo Brothers	Ranch 11	August 17, 2009	12 lines
Fall Head	D'Arrigo Brothers	Ranch 20	August 17, 2009	8 lines