CALIFORNIA LETTUCE RESEARCH PROGRAM

April 1, 2007 to March 31, 2008

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 has been a continued increase in variation in the pathogen. Of the known resistance genes, only *Dm17* remained 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 dessert 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 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. We are backcrossing to introduce resistance from 12 accessions into coastal lettuce types (Table1). Crosses have and are being made to combine DM 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 and decrease the chances that a single change in the pathogen renders multiple lettuce types susceptible.

In 2007 we conducted new germplasm screens to identify more sources of resistance. Twelve out of 95 lines screened were resistance to a range of California isolates representing the most virulent isolates currently found. Backcrossing programs to introgress the next generation of resistance to LDM new sources have been initiated (Table 1). A collection 95 lines of *L. saligna* from Professor Ales Lebeda (Palacky University, Czech Republic) will be screened for resistance this year.

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

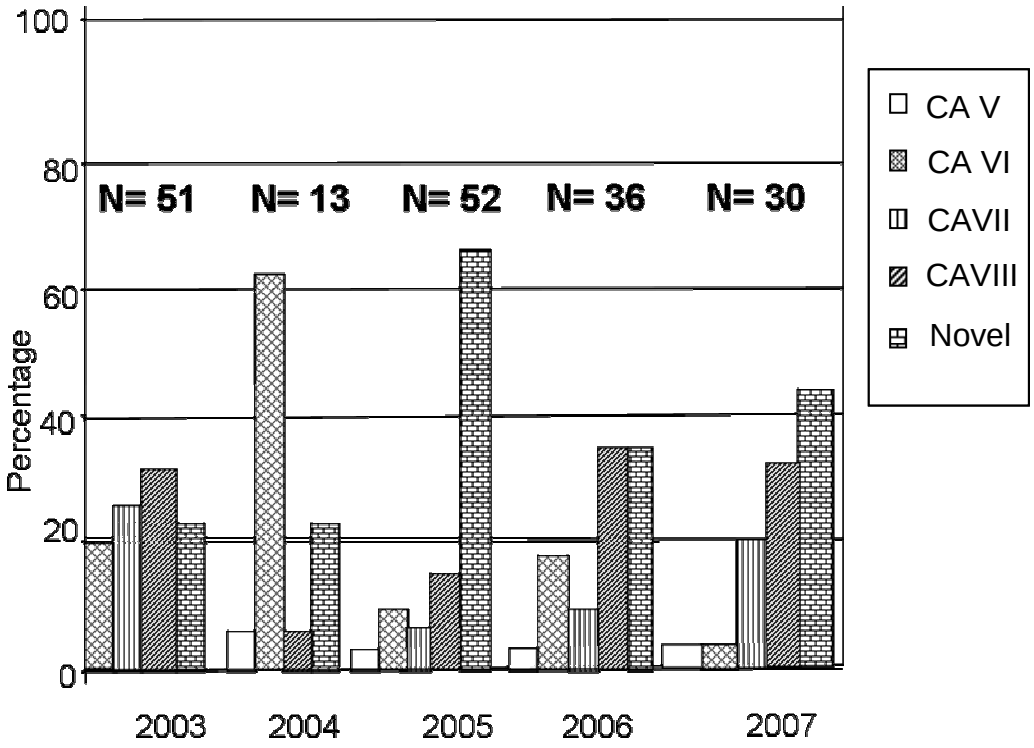
Source	*Lactuca*	Curent Status	Resistance factors
LJ-85314	*saligna*	Released 2002	33
LJ-85292	*serriola*	Released 2002	34
LJ-85289	*virosa*	Released 2002	35
W66336A	*serriola*	Released 2002	28
W66331	*serriola*	Released 2002	29
PI491226	*sativa*	Released 2008	41*
PI491108	*serriola*	Released 2008	42*
PI491206	*saligna*	Released 2008	43*
PI491208	*saligna*	Released 2008	44*
UC94Isr1	*saligna*	BC_7S_2 field trial	
CGN9311	*saligna*	BC_7S_2 field trial	
CGN5318	*saligna*	BC_7S_2 field trial	
CGN5282	*saligna*	BC_7S_1 field trial	
CGN5147	*saligna*	BC_7S_1 field trial	
UC04US2509	*saligna*	BC_2	
UC04US2507	*virosa*	BC_2	
12 lines from A. Lebeda	*serriola*	BC_1	

*: Provisional resistant factor designation.

Downy mildew virulence surveys. In order to ensure that we are breeding for resistance to current virulence phenotypes of the pathogen in California, we have continued to sample the downy mildew pathogen on an opportunistic basis with the collaboration of growers and seed

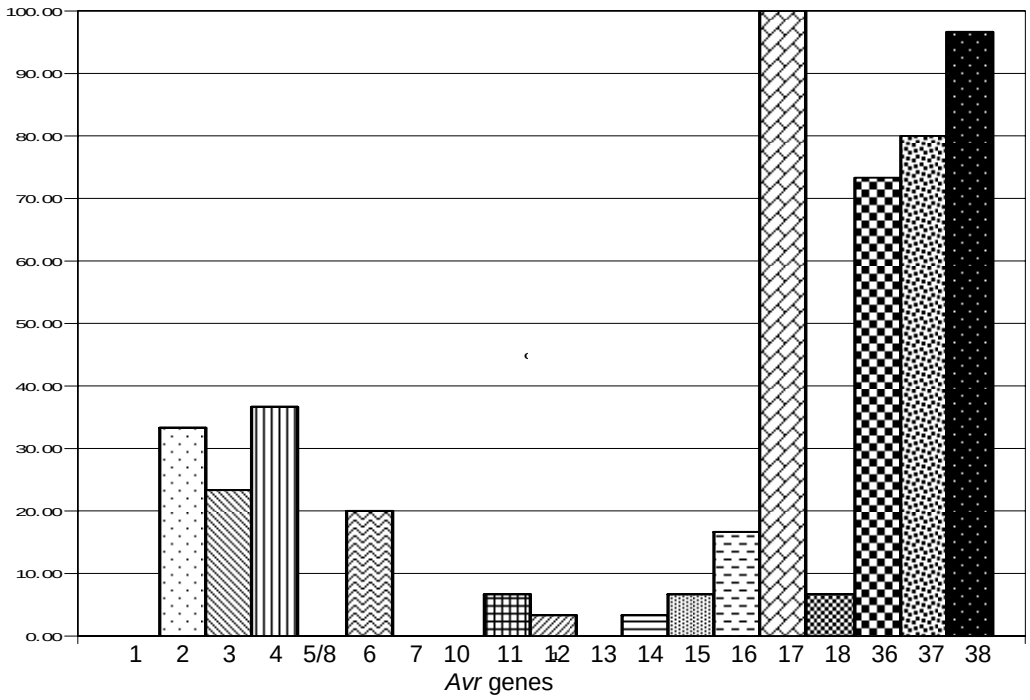
industry personnel. Over the past year, 30 isolates were characterized for virulence phenotype, fungicide sensitivity and mating type (Table 2). This opportunistic sampling provides an indication of the diversity in the field but does not provide a quantitative measure of LDM variation. 43 % were characterized as novel types. 20% and 30% of the isolates were Pathotypes CAVII and CAVIII, respectively. California pathotypes V and VI were equally rare at 3.3%.

Figure 1: Frequency of LDM pathotypes in 2003, 2004, 2005, 2006 and 2007.



All of the isolates sampled in 2007 were avirulent on *Dm17*. *Avr36*, *Avr37* and *Avr38* were detected in frequencies higher than 70% (Figure 2). The frequency of *Avr18* dropped considerably in 2007 to around 5%. *Avr4* was detected in a frequency of 38%. *Avr2*, *Avr3* and *Avr6* were again detected in the novel isolates. This is interesting because until recently avirulence to *Dm2* or *Dm3* was rarely detected in California. The presence of *Avr2* and *Avr3* may indicate that the recent novel isolates originated from a different source than isolates detected previously.

Figure 2. Frequency of avirulence genes observed in isolates analyzed in 2007.



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 avirulence phenotype of isolates in Table 2. Sextet code values were determined using the same differential series of resistant cultivars as used in the European system. 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 2007 were B_2 mating type and sensitive to the fungicides metalaxyl and Aliette. 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.

Table 3. Virulence phenotypes of isolates of *B. lactucae* analyzed in 2007.

Isolate ID	Location	Cobham Green 0	Lednicky 1 1	UC DM2 2 2	Dandie 3 3	R4T57D 4 4	Valmaine 5/8 5	Sabine 6 6	LSE 57/15 7 7	UC DM10 8 2	Capitan 9 4	Hilde II 10 8	Pennlake 13 16	UC DM14 14 32	PIVT 1309 15 1	LSE /18 16 2	LS-102 17 4	Colorado 18 8	Ninja 36 16	Discovery 37 32	Argeles 38 1	Mariska 18m 2	El Dorado 18ed 4	R32 18/32 8	Amplus 40 16	Sextetblok 1	Sextetblok 2	Sextetblok 3	Sextetblok 4	Fully avirulent	Pathotype	Mating
C07O1127	MissionRanch Lab	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-	63	63	11	14	17,36,37,38,40	CAVIII	B2
C07O1128	Davis, Ca	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	-	63	63	43	15	17,36,38	CAVIII	B2
C07O1129	Spence, CA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	-	63	63	27	14	17,37,38,40	CAVIII	B2
C07O1130	Spence, CA	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+	-	+	+	+	+	25	63	43	30	2,3,6,17,36,38	Novel	B2
C07O1131	Spence, CA	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+	-	+	+	+	+	25	63	43	30	2,3,6,17,36,38	Novel	B2
C07O1132	Spence, CA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	-	63	63	27	14	17,37,38,40	CAVIII	B2
C07O1133	Salinas, CA ?	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+	-	-	+	+	+	29	63	43	28	2,6,17,18m,36,38	Novel	B2
C07O1134	Watsonville,CA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	-	-	63	63	11	4	17,36,37,38,18m,40	CAVI	B2
C07O1135	Gonzales, CA	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-	55	63	59	14	4,17,38	CAVII	B2
C07O1136	Gonzales, CA	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	+	-	55	63	27	12	4,17,37,38,18m	CAV	B2
C07O1137	Gonzales, CA	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	55	63	27	30	4,17,37,38	CAVII	B2
C07O1138	Salinas, CA	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	+	+	55	63	27	28	4,17,37,38	CAVII	B2
C07O1139	Watsonville,CA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-	63	63	11	14	17,36,37,38,40	CAVIII	B2
C07O1140	Watsonville,CA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-	63	63	11	14	17,36,37,	CAVIII	B2
C07O1141	Watsonville,CA	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+	29	63	11	30	2,6,17,36,37,38	Novel	B2
C07O1142	?	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-	63	63	11	14	17,36,37,38,40	CAVIII	B2
C07O1143	?	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+	29	63	11	30	2,6,17,36,37,38	Novel	NT
C07O1144	?	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+	63	63	11	30	17,36,37,38,40	CAVIII	B2
C07O1145	?	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+	63	63	11	30	17,36,37,38	CAVIII	B2
C07O1146	Spence, CA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	63	63	59	30	17,38	CAVIII	B2
C07O1147	Spence, CA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	63	63	59	30	17,38	CAVIII	B2
C07O1148	Spence, CA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	63	63	59	30	17,38	CAVIII	B2
C07O1149	Spence, CA	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	(-)	(-)	-	63	63	0	0	15,16,17,18,36,37,38,40	Novel	B2
C07O1150	Spence, CA	+	+	-	-	-	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-	+	+	+	-	49	19	1	14	2,3,4,11,12,14,15,16,17,18,36,37,38	Novel	B2
C07O1151	Spence, CA	+	+	-	-	-	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	+	+	+	-	49	63	10	14	2,3,4,15,17,36,37,38	Novel	B2
C07O1152	Salinas, CA	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	+	-	-	-	+	-	+	-	51	59	11	10	3,4,11,17,36,37,38,40	Novel	B2
C07O1153	Salinas, CA	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+	+	-	61	63	9	14	2,16,17,36,37,38	Novel	B2
C07O1154	Salinas, CA	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	-	-	57	63	43	0	2,3,17,36,38,18m,18ed,18/32,	Novel	B2
C07O1155	Salinas, CA	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	(-)	(-)	-	55	63	27	0	4,17,37,38,40	CAVII	B2
C07O1156	Salinas, CA	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+	+	-	23	63	9	14	4,6,16,17,36,37,38	Novel	B2
C07O1157	Salinas, CA	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	(-)	-	-	-	-	-	(-)	55	63	11	0	4,17,36,37,38	CAVII	B2
C07O1158	Salinas, CA	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+	+	-	55	63	9	14	4,16,17,36,37,38	CAVII	B2
C07O1159	Salinas, CA	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	57	63	11	0	2,3,17,36,37,38	Novel	B2

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 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 Subarao (UC Davis). We include Salinas as the susceptible control genotype with La Brillante representing a genotype that has shown less disease in the field (K. Subarao, pers. comm.).

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

In 2007 we screened 95 lines of *Lactuca serriola* of diverse geographical origin provided by Professor Ales Lebeda (Palacky University, Czech Republic) for resistance to isolate Vdls17 using the greenhouse microplots. These included 22 lines collected from the Czech Republic, 15 from Germany, 8 from The Netherlands, 10 from the UK, 4 from Austria, 5 from Italy, 3 from France, 9 from Slovenia, 12 from Sweden, 5 from the USA, and 1 from New Zealand. Six plants were tested for each accession. All of the lines screened developed severe disease and none showed resistance to Verticillium race 2. The few plants with low disease index ratings that were able to produce some seeds were screened for seed transmission by plating them on PDA. Out of those with very low transmission, a sample was replanted, reinoculated, monitored for symptoms and assayed by PCR for Verticillium; all of these lines showed symptoms after reinoculation and Verticillium was detected by PCR. Therefore no fully resistant accessions were identified. Additional results on Verticillium resistance are presented in the CLRP Leafy report.

Over the past three years, a total of 267 accessions of *L. sativa* (21), *L. serriola* (183), *L. saligna* (48) and *L. virosa* (15) from several collections have been screened for resistance to Verticillium wilt in the microsclerotia-infected microplots in the greenhouse. We have now screened nearly all the germplasm that is readily available to us. Acquisition of new germplasm to screen is a high priority. A disease severity index (DSI) was prepared for each accession as described in previous reports and samples were taken for PCR to assess the amount and location of Verticillium in the plant. Some accessions were highly susceptible to isolate VdLs17, some had intermediate responses and some showed reduced or delayed symptoms. However, no fully resistant accession has been detected so far. Screening of additional germplasm continues. Currently we are evaluating 66 accessions of *L. saligna* from Israel, Italy, France, Turkey, Portugal and Jordan provided by Ales Lebeda for resistance to Vdls17 (race2).

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.

We have selected plants that exhibited reduced symptoms in the germplasm screens and inter-crossed them. 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 slection.

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 mapping and breeding populations will be informative for resistance to Verticillium. In collaboration with Dr. Ryan Hayes (USDA), we will analyze the RIL (Recombinant Inbred Line) population from La Brillante x Salinas 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 in the Leafy Breeding Report.

Multiple Disease Resistance

Crosses between advanced breeding lines, field selections, and released lines have been made to generate lines with multiple disease resistances. Screening for multiple diseases, 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 selected at field trails in collaboration with Richard Smith (UC Monterey Cooperative Extension, Salinas). Backcross or modified single-seed descent strategies are being employed for most of the early generations. The first Salinas field trial was planted in March 2007 at the Jacop Ranch, Boutonnet Farms. The second and third trials were planted at the Panziera Ranch, Royal Farms in June 2007 and in August 2007 respectively. We continue to select for good color, slow bolting, and yield as well as disease resistance in Salinas plant types.

Releases of Downy Mildew Resistant Lines

We released four advanced crisphead breeding lines in March 2008 (see release note at the end of report). Sixteen different seed companies and research groups have so far requested these releases. 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 personal from the seed industry and others to handle lettuce downy mildew, corky root, anthracnose and other diseases.

RELEASE OF DOWNY MILDEW RESISTANT BREEDING LINES OF CRISPHEAD LETTUCE

Please contact Oswaldo Ochoa (oeochoa@ucdavis.edu) or Richard Michelmore (rwmichelmore@ucdavis.edu) to receive samples of these lines.

Advanced breeding lines of crisphead lettuce are available for use by plant scientists and breeders in public and private institutions. These lines were developed by Oswaldo Ochoa, Maria Jose Truco and Richard Michelmore at University of California, Davis. Feedback as to the utility of these lines and other material from the UC Davis lettuce genetics and breeding program is always welcome. When this germplasm contributes to a new cultivar, appropriate recognition should be given as to its origin.

These lines have been developed to provide superior disease resistance to downy mildew in a Salinas horticultural type by backcrossing wild resistant accessions with cv. Salinas as the public domain recurrent parent. To our knowledge, these sources of downy mildew resistance have not been used as parents for existing cultivars grown in California and therefore provide new genes for resistance to downy mildew. Resistant individuals were selected in each generation. These lines are now homozygous for resistance to all Californian isolates tested. Different genes have been introgressed into each type to fragment the selection pressure on the downy mildew pathogen. These genes are also different from those introgressed into the leafy and romaine types. This may increase the durability of the individual resistance genes and prevent virulent variants causing epidemics on multiple horticultural types. These resistance genes will, however, probably be rendered ineffective over time due to changes in the pathogen.

To the extent possible, the Salinas plant type was selected in each generation. Field evaluations in Salinas were made during the last two selfed generations. These lines are close to horticultural types suitable for use in the coastal production areas of California. However, there is residual variation in most of these lines and further selections are required to fix plant type. Trails and selections should be made to determine specific areas and seasons to which these lines are best adapted. Crosses to lines of the same horticultural type are encouraged to provide these resistances in genotypes adapted to additional areas and seasons.

Table1: Reactions of releases to pathotypes of downy mildew.

					Rxn to California Pathotypes			
Designation	Donor	*Lactuca*	Generation	Resistance	CaV	CaVI	CaVII	CAVIII
UC07105	PI491226	*serriola*	$BC_7 S_2$	R*	-	-	-	-
UC07106	PI491108	*serriola*	$BC_7 S_2$	R*	-	-	-	-
UC07107	PI491206	*saligna*	$BC_7 S_2$	R*	-	-	-	-
UC07108	PI491208	*saligna*	$BC_7 S_2$	R*	-	-	-	-

*: Novel resistant genes to be assigned after further genetic analysis. - = resistant reaction.