CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

April 1, 2017 to March 31, 2018

PROJECT TITLE: **BREEDING CRISPHEAD AND LEAFY LETTUCE**

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CONTINUING OBJECTIVES:

1) To identify new genes for disease resistance in wild germplasm and incorporate multiple genes from diverse sources into advanced crisphead and leafy breeding lines to maximize the likelihood of durable resistance.

2) To monitor variation in pathogen populations, particularly downy mildew, to facilitate the deployment of effective resistance genes.

3) To utilize the genetics of agriculturally important traits, particularly disease resistance.

4) To release advanced crisphead and leafy 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

Breeding Strategy

The overall strategy used in the UC Lettuce Breeding Program continues to be to initiate crosses and grow early generations at Davis; later generations are trialed and selected at several different lettuce-growing areas in collaboration with USDA, Cooperative Extension in Salinas, and California growers. Backcross and/or single-seed descent strategies are employed for most of the early generations. We select for type, color, slow bolting, and yield as well as disease resistance in the crisphead and the four leaf lettuce plant types. As far as possible we use different sources of resistance for each plant type. 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.

When resistant advanced lines of the desired plant type have been generated for individual resistances, they are intercrossed to create lines with multiple disease resistances for lettuce downy mildew (LDM), corky root (CR), anthracnose (ANT), lettuce mosaic virus (LMV), and *Verticillium* race1 (*Ve1*). Additionally, we have screened germplasm to identify genetic resistances for *Verticillium* race 2 and *Fusarium* wilts, which are being incorporated into the breeding program.

Cultivars representing each type were selected to be the recurrent parents in backcross programs to introgress resistance genes based on their horticultural type and performance in California, their status in the public domain, and the presence of additional disease resistance genes. We are currently using Salinas, Green Towers, Tropicana, Red Fox, Red Tide, Bibb and Buttercrunch for the crisphead, romaine, green leaf, red leaf, and butterhead programs, respectively (Table 1). We welcome suggestions and further input on the field performance of these and other potential recurrent parents. The recurrent parents used in the final generations of backcrossing will be adjusted to reflect the industry standards as they advance.

Introgression of Resistance to Downy Mildew

We are continuing to develop crisphead and leafy lettuce lines with resistance genes from diverse sources to provide protection against downy mildew in California. We are focused on generating advanced breeding lines with new resistance genes identified through germplasm screens in previous years (Table 1). Mapping and characterization of the new sources are in progress to provide a pipeline of new genes for resistance. Backcrossing programs to introgress the next generation of genes for resistance to DM into crisphead and leafy types is a continual ongoing process.

Table 1: Status of introgression of new sources of DM resistance into crisphead and leafy types.

Donor	*Lactuca*	Type	Status	Category
PI491000	*L. saligna*	Green Leaf	BC_7S_1	Advanced
05G1411	*L.serriola*	Green Leaf	BC_7S_1	Advanced
ISR-380	*L. serriola*	Romaine	BC_7	Advanced
CGN5309	*L. serriola*	Green Leaf	BC_5	Advanced
CHEC-023	*L. serriola*	Iceberg	BC_6	Advanced
CHEC-063	*L. serriola*	Iceberg	BC_6	Advanced
CHEC-076	*L. serriola*	Iceberg	BC_5	Advanced
RUSS-635	*L. serriola*	Red Leaf	BC_5	Advanced
KYRGY-237	*L. serriola*	Romaine	BC_5	Advanced
GEOR-289	*L. serriola*	Romaine	BC_5	Advanced
GEOR-297	*L. serriola*	Red Leaf	BC_5	Advanced
CHEC-147	*L. saligna*	Romaine	BC_4	Intermediate
CHEC-101	*L. saligna*	Romaine	BC_4	Intermediate
CHEC-136	*L. saligna*	Red Leaf	BC_4	Intermediate
GEOR-301	*L. serriola*	Romaine	BC_4	Intermediate
GEOR-292	*L. serriola*	Green Leaf	BC_4	Intermediate
GEOR-299	*L. serriola*	Iceberg	BC_4	Intermediate
CHEC-022	*L. serriola*	Iceberg	BC_4	Intermediate
CHEC-075	*L. serriola*	Iceberg	BC_4	Intermediate
CHEC-082	*L. serriola*	Iceberg	BC_4	Intermediate
CHEC-083	*L. serriola*	Iceberg	BC_3	Intermediate
CHEC-088	*L. serriola*	Iceberg	BC_4	Intermediate
CHEC-089	*L. serriola*	Iceberg	BC_4	Intermediate
05G1421	*L.serriola*	Butterhead	BC_4	Intermediate
CHEC-158	*L. saligna*	Green Leaf	BC_3	Intermediate
CHEC-132	*L. saligna*	Green Leaf	BC_3	Intermediate
KYRGY-247	*L. serriola*	Butterhead	BC_2	Intermediate
CHEC-108	*L. saligna*	Green Leaf	BC_2	Intermediate
CGN13330	*L. saligna*	Romaine	BC_1	Early
RUSS-653	*L.serriola*	Butterhead	BC_1	Early
GEOR-282	*L. serriola*	Butterhead	BC_1	Early
GEOR-284	*L. serriola*	Red Leaf	BC_1	Early
GEOR-288	*L. serriola*	Iceberg	BC_1	Early
CGN5157	*L. saligna*	Green Leaf	BC_1	Early
ARM09-158	*L. serriola*	crisphead	BC_1	Early
ARM09-169	*L. serriola*	crisphead	BC_1	Early
ARM09-172	*L. serriola*	crisphead	BC_1	Early
CGN5301	*L. saligna*	romaine	F_1	Early
AZER-805	*L.serriola*	Red Leaf	BC_1	Early
AZER-811	*L.serriola*	Romaine	BC_1	Early
AZER-822	*L.serriola*	Red Leaf	BC_1	Early
AZER-840	*L.serriola*	Romaine	BC_1	Early
AZER-843	*L.serriola*	crisphead	BC_1	Early
AZER-853	*L.serriola*	Green Leaf	BC_1	Early
AZER-854	*L.serriola*	Green Leaf	BC_1	Early
AZER-871	*L.serriola*	Butterhead	F_1	Early

Each line is tested in each generation against a current, highly virulent isolate and the resistant progeny used as the resistance donor parent for the next generation. Selfed generations of advanced lines (BC_6S_1) are then tested for resistance to a diverse panel of contemporary virulent Californian isolates. Susceptible lines are discarded. We are genetically characterizing the chromosomal position of all of our resistance genes. Once these genomic regions are identified, molecular markers will be used for MAS for selection of lines with multiple resistance genes that confer resistance to all Californian isolates. Release of lines with pyramids of several resistance genes should increase the durability of the individual genes and provide longer lasting resistance.

Downy Mildew Surveys

In order to ensure that we are breeding for resistance against virulent phenotypes of the pathogen *Bremia lactucae* currently present in California, we have continued to sample *B. lactucae* in collaboration with agricultural and seed companies, growers, and extension personnel. We have analyzed isolates of downy mildew since 1982. On average ~50 isolates have been characterized per year with a total of more than 2,010 isolates characterized to date. In most years the sampling was opportunistic; this provided a qualitative rather than a quantitative understanding of the phenotypes of isolates in the field. From September 2013 to June 2016, we were funded by the California Department of Food and Agriculture to conduct a detailed characterization of variation of *B. lactucae* in California. During this period the number of isolates characterized averaged ~180 isolates per year. This provided a comprehensive understanding of variation in California. Since the end of this funding, we have had to return to opportunistic characterization of lower numbers of isolates.

We maintain the *Bremia* database to display the virulence of California isolates characterized by us and others (http://bremia.ucdavis.edu/bremia_database.php). This database has information on the origin, virulence phenotype, mating type and fungicide sensitivity of isolates dating back to 2001. Isolates are entered as their phenotypes are characterized so that collaborators, pest control advisors, and others can access the data as soon as it is generated; however, this is still several (4 to 8) weeks after an isolate is collected due to the need to increase each isolate prior to inoculation and scoring on the differential series of resistance cultivars. Subject to funding, we aim to develop PCR-based that will allow more rapid characterization.

In 2014 the American *Bremia* Evaluation Board (ABEB) was initiated by interested breeding companies and UC Davis. This is a breeding company coordinated group that will nominate official Pathotypes for *B. lactucae* in the western US using a similar protocol to that used in Europe by the International *Bremia* Evaluation Board (IBEB). In May 2015, there was a joint meeting of ABEB and IBEB and it was decided to coordinate the activities of both groups under a single IBEB-G(lobal) umbrella with two sub-groups IBEB-EU and IBEB–US responsible for activities in Europe and the US, respectively. Both groups now use the same core differential set of resistant cultivars so that data can be compared. Nomenclature has been standardized so that denominated isolates are designated Bl:#EU or US. The US Pathotypes I to VIII are now designated as Races Bl:1US to Bl:8US. When isolates of the same virulence phenotype are observed in multiple years and locations in California that overcome important *Dm* genes, they will be

nominated by IBEB-US for designation as a new Race (see below for Race Bl:9US). A reference isolate will be distributed to the companies to confirm the phenotype and its stability; if confirmed, it will be designated as an official Race and used in cultivar resistance descriptions. There are currently nine official Pathotypes/Races; however, Races Bl: 1US to Bl: 4US have not been observed for many years; therefore they are not of agricultural relevance and are not available for distribution and screening. Individuals interested participating in IBEB-US should contact Nicki Phillips (nicki.phillips@enzausa.com).

Over the past year, 71 isolates of *B. lactucae* mostly representing opportunistic samples provided by collaborators from several regions in California were characterized for virulence phenotype, mating type, and metalaxyl sensitivity. None of the isolates analyzed were Race Bl: 5US or 6US; 7% were 7US; 14% were 8US; and 35% were candidate Race Bl: US9 progenitor (see below). By far the majority of isolates (44%) had novel virulence phenotypes that differed from the designated races (Fig. 1). Of all the isolates in 2017, only ten (22%) were able to overcome *Dm17*. *Avr36* and *Avr38* were present in 35 and 64% of the isolates, respectively. *Avr4* was detected at a frequency of 22%; *Avr6* had increased to 89% of isolates (Fig. 2). The new inclusions in the differential set RYZ2164 (n1), RYZ910457 (n2), Bedford (n3), Balesta (n4) and Bellissimo (n5) showed resistance; of these, Balesta (100% of isolates avirulent) and Bellissimo (88% of isolates avirulent) were the most effective against the isolates tested.

Figure 1: Frequency of downy mildew Pathotypes detected in CA 2010-2018

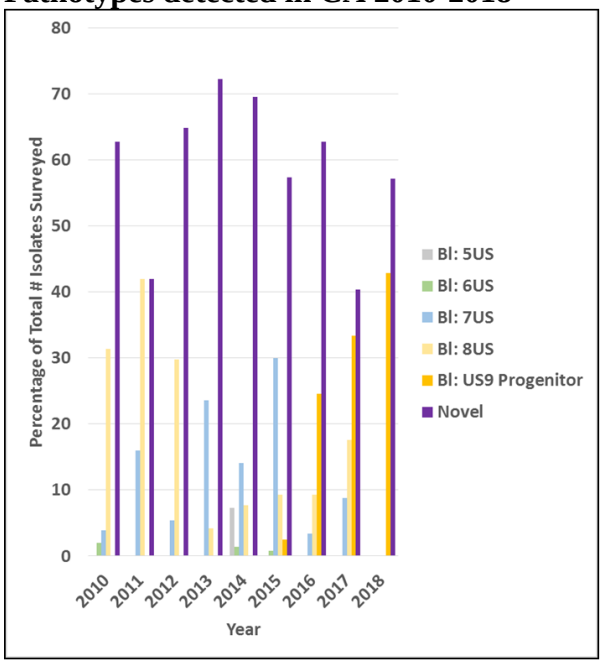
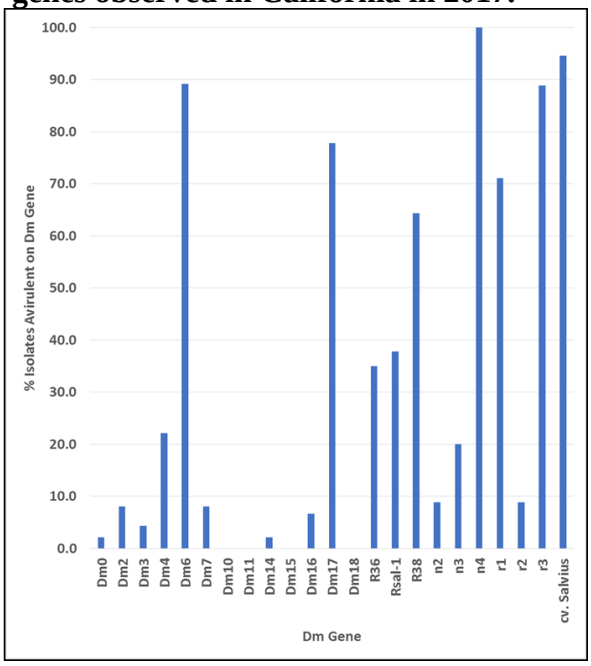


Figure 2: Frequency (%) of avirulence genes observed in California in 2017.



From 2009 to 2015 we identified 298 isolates with novel virulence phenotypes. Of these 49 were avirulent on *Dm3*, *Dm6*, *Dm17*, *Dm36*, and variable on *Dm37* and *Dm38*. This group of novel isolates was nominated as a candidate for Pathotype CAIX (Bl: 9US)

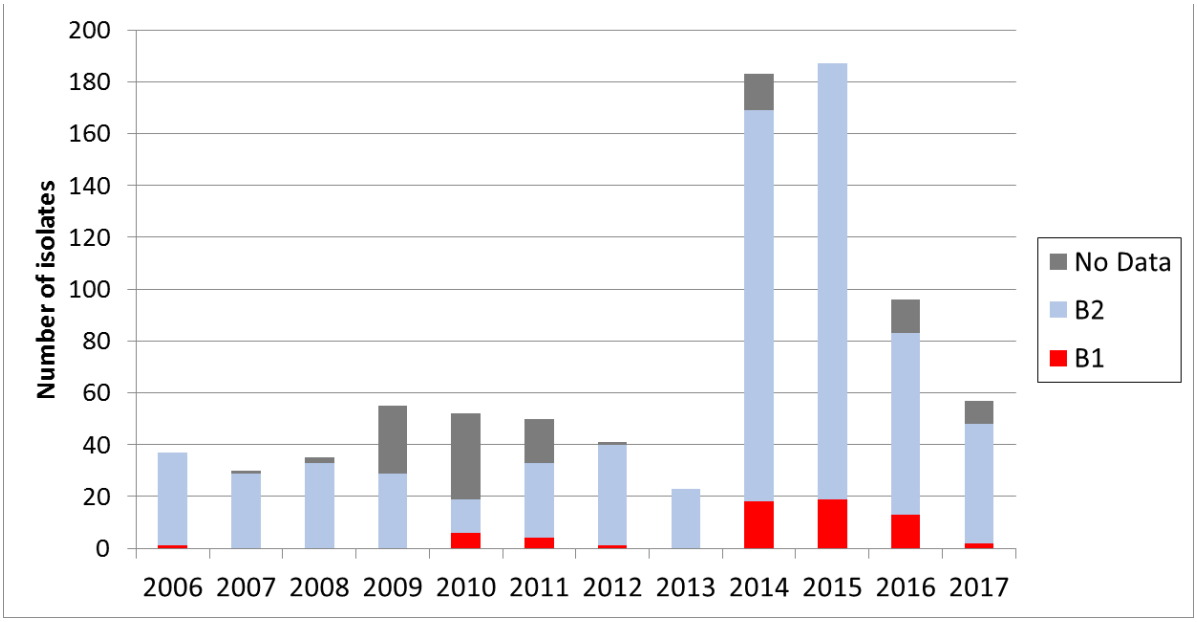
because they have similar virulence phenotypes, had been detected in multiple locations and over multiple years, and overcome important *Dm* genes. A candidate type isolate for Race Bl: 9US was distributed to seed companies for testing; however, its virulence phenotype was not stable. Furthermore, the isolates with this virulent phenotype were not observed in the field. Therefore this isolates was removed from consideration as Bl: 9US.

In 2016, another candidate for race Bl: 9US with a different virulence phenotype was submitted for ring testing. This was avirulent on *Dm6*, 17/n1, 38, n4, r1, r3 and was considered important because of its ability to infect FrSAL-1. However, initial ring tests indicated that it was unstable. After several rounds of passaging on FrSAL-1 to stabilize that phenotype, derived isolates were retested for virulence phenotype by UCD and the companies. The serial passaging on FrSAL-1 changed this isolate to avirulence on *Dm4*, 38, n1/17, n4, r1, r3. Although this virulence phenotype is rarely observed in the field, this isolate was accepted as the designated race Bl: 9US to allow for screening for resistance to a stable isolate. The isolate's virulence phenotype prior to passaging on FrSAL-1 remains a common phenotype observed in the field and is referred to as Bl: 9US-progenitor (Fig. 1).

No new candidate type isolates are currently undergoing ring tests.

Of 48 isolates characterized for mating type in 2017, 46 (96%) and 2 (4%) were B_2 and B_1 respectively. The frequency of isolates of B_1 mating type varied during the years (Fig. 3) with higher frequencies between 2014 and 2016.

Figure 3: Frequency of isolates with B_1 or B_2 mating types between 2006 and 2017.



Of the 57 isolates analyzed for metalaxyl sensitivity in 2017, 41 (72%), 12 (21%), 2 (3.5%), and 2 (3.5%) were insensitive, delayed insensitive, intermediate, and sensitive respectively. Insensitivity is considered as the ability to sporulate by 15 dpi at 50 ppm or above. An intermediate reaction is considered as sporulation by 15 dpi at 5 or 10 ppm but not at 50 ppm. Sensitivity is considered as no sporulation by 15 dpi at 5 ppm or above. Some isolates were highly insensitive and could sporulate on seedlings growing in 1,000

ppm metalaxyl. There was no obvious correlation of metalaxyl sensitivity with virulence phenotype.

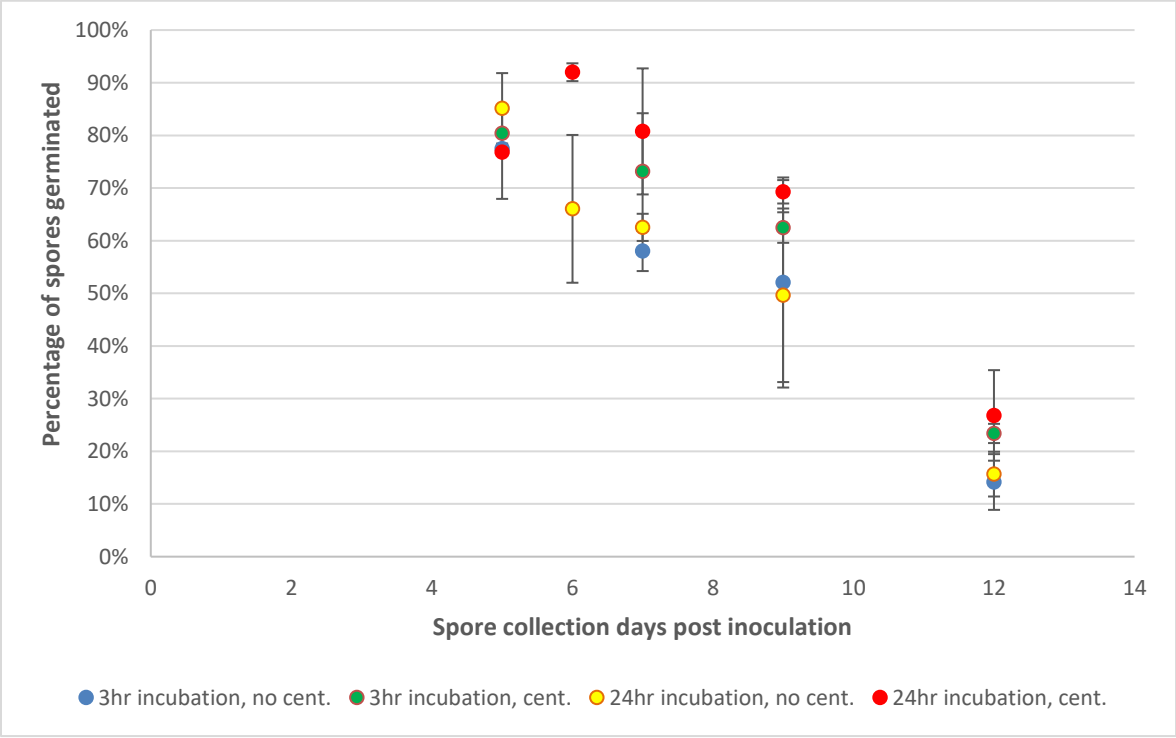
The importance of potential variables in tests for virulence phenotype.

Spore germination tests were made to investigate potentially important factors that might cause differences in virulence phenotyping results obtained by different labs.

Effect of centrifugation and age on spore viability

We investigated the importance of centrifugation of the suspension of spores to remove a potential inhibitor of germination and whether spore age affected viability. There had been a report that centrifugation was necessary to remove a water soluble inhibitor of germination; however, this had not been carefully investigated. Also, the spores of some downy mildews of other crops germinate rapidly and therefore collecting after several days of sporulation might result in lower numbers of viable spores. Sporulation started 5 days post inoculation (dpi). We collected three replicates of spores of isolate 622b of various ages between 5 and 12 dpi in distilled water. Half of each sample was centrifuged and resuspended in fresh water. The remaining spores were kept in the water used for collection. Suspensions of spores were placed on glass slides at ~1 x10^5 and incubated in a moist dish at 15^{o}C in an illuminated growth room (12 hours light per day, at least 5 hours light followed by 12 hours dark). Viability was assessed as the percentage of germinated spores after 3 and 24 hours of incubation.

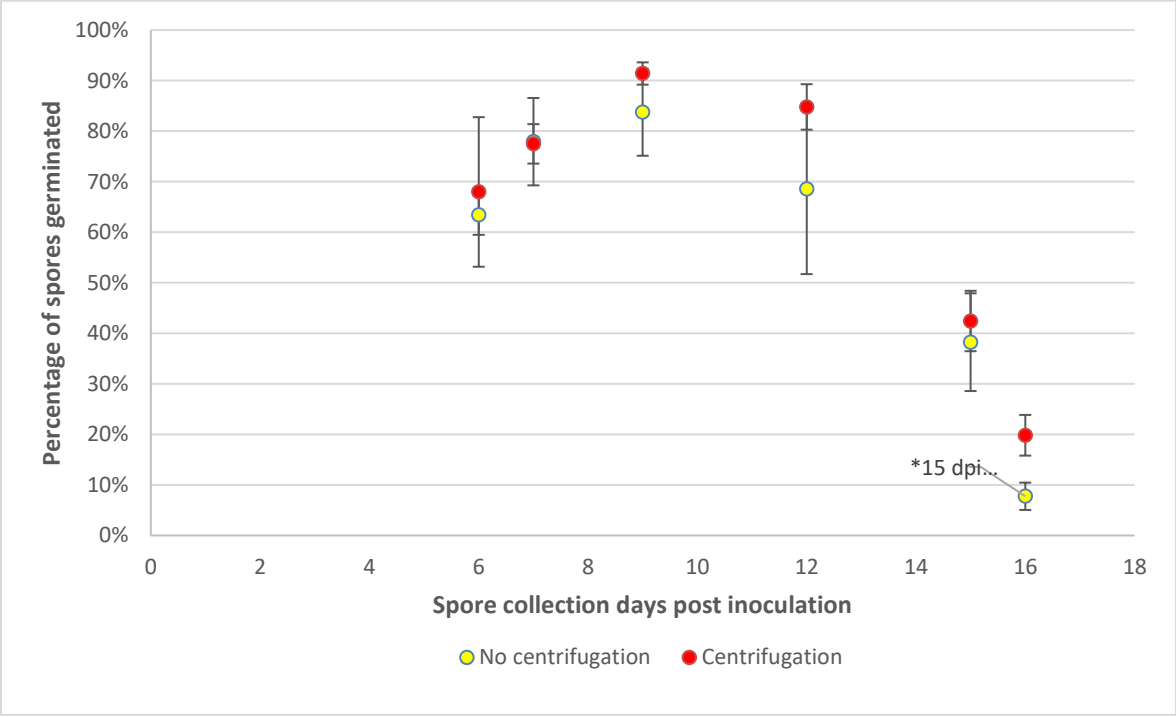
Figure 4: Germination of *Bremia* spores 3 and 24 hours after collection, with and without centrifugation. The error bars show the s.d. of three replications.



The majority of spores were germinating after 3 hours of incubation at 15ºC (within ~4 hours of collection). Centrifugation had no significant effect on viability (Fig. 4). Therefore there was no evidence of a water soluble inhibitor of germination and consequently centrifugation is not considered an important step in the protocol. Spore germination decreased with increasing dpi (Fig. 4). This was probably at least in part due increasing numbers of bacteria as the seedlings began to decay.

To test whether increasing numbers of bacteria caused the observed reduction in germination, the experiment was repeated. Spores were again collected at several dpi. They were also collected from seedlings that had been shaken in water to collect spores 15 dpi; these spores were therefore less than 24 hours old. The frequency of germination again decreased with increasing dpi, although the curve was shifted to the right (Fig. 5). The germination of the young spores collected 16 dpi was very poor consistent with bacteria causing the reduction in germination (Fig. 5). This emphasizes the need to collect spores from seedlings with fresh sporulations rather from seedlings or leaf lesions with decaying tissue. The consistently slightly lower rates of germination in samples that had not been washed by centrifugation may reflect the removal of some bacteria by the centrifugation procedure, indicating that pelleting by low speed centrifugation may be beneficial when the plant samples are beginning to decay.

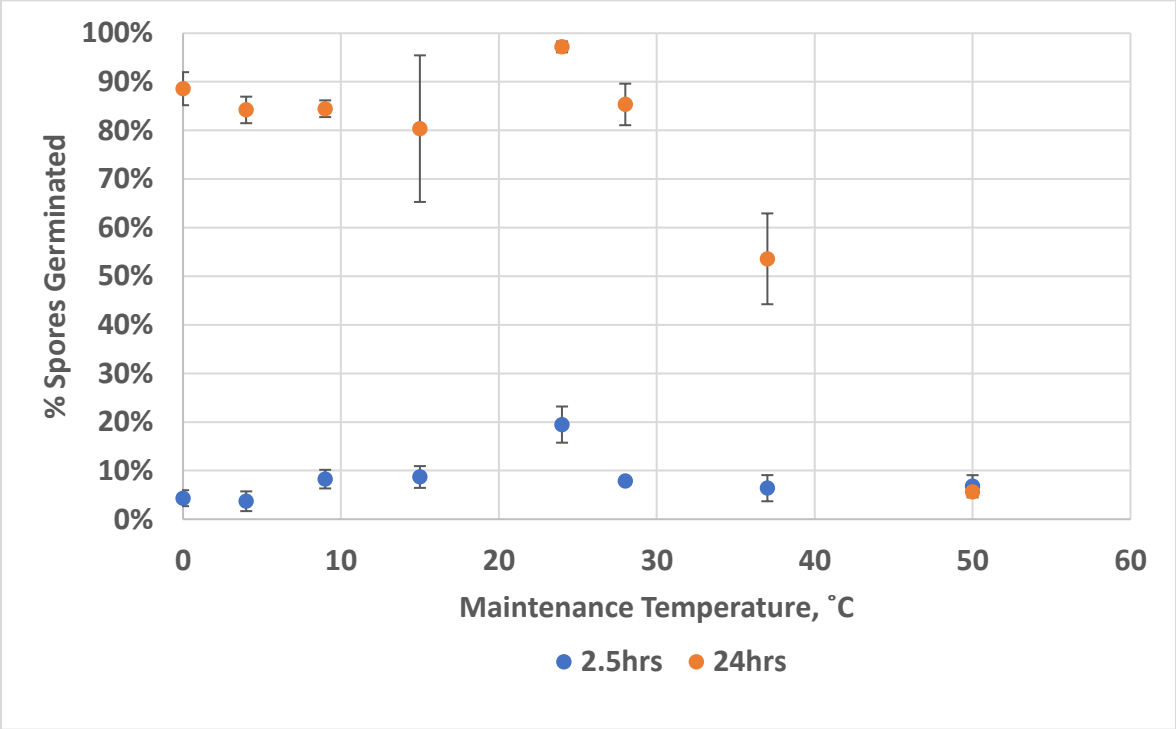
Figure 5: Germination of *Bremia* spores of varying age observed 24 hours after collection. Spores were recollected from 15 dpi seedlings and observed for germination at 16 dpi. The error bars show the s.d. of three replications.



Effect of collection temperature on spore viability

We also investigated whether maintenance at different temperatures immediately after collection affected the subsequent frequency of germination at 15ºC. Spores of isolate 622b were collected in water and maintained at different temperatures ranging from 0 to 50 °C for 2.5 hours (representing the maximum time that spores might be kept before inoculation onto seedlings). They were observed for germination before being moved to 15°C. After 24 hours of incubation at 15°C, the same spores were again observed for germination. Surprisingly, there were no large differences in germination among spores maintained at temperatures below 30°C. Only incubation temperatures above 35°C reduced germination (Fig. 4). Therefore *Bremia* spores do not seem to be highly sensitive to temperature prior to inoculation.

Figure 6: Germination of LDM spores maintained at different temperatures for 2.5 hrs and then shifted to 15°C for 24 hrs. The error bars show the s.d. of three replications.



Resistance to *Verticillium* wilt

Resistance to *Verticillium* wilt is a high priority for our program. We previously developed an efficient, reliable, and contained method for screening for the reaction of lettuce to *V. dahliae* in the greenhouse. We utilize microplots within the greenhouse with restricted access to minimize the opportunity for spread of the pathogen (see previous reports). We continue to screen for resistance to *V. dahliae* strain VdLs17 (race 2) provided by Dr. Krishna Subbarao. We include cv. Salinas as the susceptible control genotype with cv. La Brillante representing a genotype that has resistance to race 1. In 2016 we received 67 new accessions of *L. serriola* from Azerbaijan; these were seed increased in 2017 and are currently being screened for resistance in the greenhouse (Fig.7).

Figure 7. The ongoing 2018 trial of 67 lines from Azerbaijan for resistance to *Verticillium* race 2.

The majority of accessions screened for resistance over the past six years have been highly susceptible to race 2, although differences in symptoms have been observed. A few accessions of *L. serriola* showed delayed development of symptoms or were asymptomatic. In particular, seven lines from Armenia showed no symptoms when initially screened against VdLs17 (race 2) and exhibited no seed transmission. We developed a RIL population from a cross between *L. sativa* PI251246 that is highly susceptible to *Verticillium* race 2, and *L. serriola* acc. Arm09-170-1-5 that was asymptomatic. These lines have similar vegetative development patterns and flowering times. A QTL for resistance was identified in linkage group 6 that was in significant trials conducted in Davis and Salinas. The same QTL was significant in other populations (collaboration with German Sandoya; now at University of Florida). We are introgressing this QTL for resistance to *Verticillium* race 2 into cultivated lettuce.

Resistance to *Fusarium* Wilt

We have continued our genetic analysis of resistance to *Fusarium oxysporum* race 1 in collaboration with Dr. Thomas Gordon (Dept. Plant Pathology, UC Davis). We previously developed four populations involving resistant (R), partially resistant (PR) and susceptible (S) cultivars: Salinas (PR) x Green Towers (R), Lolla Rosa (R) x Salinas (PR), Lolla Rosa (R) x Green Towers (R) and Lolla Rosa (R) x Red Tide (S) (R = resistant, PR = partially resistant, S = susceptible) and genetically characterized the resistance to *Fusarium*

in these populations. In collaboration with Jim McCreight, we also phenotyped an F_2 population generated from a cross between King Louie (R) x Autumn Gold (S) for *Fusarium* resistance. Different QTLs were identified for resistance to *Fusarium* in these five populations. This provides the possibility of combining multiple sources of resistance in a single genetic background. Only the top of LG4 had a QTL identified in two populations. Fine mapping of the QTL regions is underway.

The best ~20 resistant lines have been selected based on previous field data from each of Valmaine x Salinas, Salinas x Green Towers, Lolla Rosa x Salinas and Red Tide x Lolla Rosa populations in order to select *Fusarium* resistant lines of different lettuce types. These lines are being characterized genetically to identify molecular markers associated with the QTLs for resistance prior to release to seed companies.

Supply of Isolates

We have continued to supply current California isolates of downy mildew and corky root to breeding companies and other research groups. In particular, we have supplied isolates of the nominated Races Bl:5US to Bl:8US as well as candidate isolates for ring tests. We have trained personnel from the seed industry and others to handle lettuce downy mildew.