California Leafy Greens Research Program
Final Report
April 1, 2013 to March 31, 2014

Project Title: Development of management strategies for Bacterial Leaf Spot of Lettuce.

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ABSTRACT

Xanthomonas campestris pv. *vitians* strains causing bacterial leaf spot of lettuce differ genetically and pathogenically on lettuce. Previously five genotypes of the pathogen were identified. Research conducted to date indicates that virulence on resistant cultivars is correlated to the pathogen genotype and strains from three genotypes (B, D, and E) were controlled by resistance conferred by the HR in Little Gem, Pavane and La Brillante whereas all members of genotypes A and C tested to date overcome this resistance. Recently identified resistance from *Lactuca serriola* was effective against all strains from genotype A but was not effective against strains from genotype C. A sixth genotype has been identified and will be included in further screening. Additional screening for lettuce germplasm resistant to genotype C will be undertaken. Publications and germplasm releases discuss novel germplasm with resistance to BLS evaluated as part of this project.

OBJECTIVES

A. Provide BLS phenotype evaluations for Lettuce Breeding objectives.
B. Screen bacterial strains available in our collection for their ability to overcome resistance to bacterial leaf spot currently deployed.
C. Collect and monitor strains of *Xanthomonas campestris* pv. *vitians* isolated from diseased lettuce throughout California for ability to overcome resistance.

PROCEDURES

A. Provide BLS phenotype evaluations for Lettuce Breeding objectives.

Resistance conferred by all mechanisms was evaluated using spray inoculations followed by disease assessment using modifications of previously published methods (Bull et al., 2007). Lettuce cultivars, RILs, PI lines or *L. serriola* accessions were planted in potting mix in 2 x 2 cm square cells in 11 x 15 cell flats. Flats were incubated at 10°C for 2 days in the dark followed by incubation in the greenhouse. Lawns of *X. campestris* pv. *vitians* BS347 and/or other strains were prepared using a spectrophotometer adjusted to 0.600 OD at 600nm in phosphate-buffer. The inocula were sprayed using a hand held spray bottle onto the leaves of three-week-old plants until run-off. Sterile 0.01 M phosphate buffer (pH 7.0) was used as a control. Plants were incubated in the greenhouse misting room (26°C, 100% RH) for a total of 21 days. Inoculated

leaves were evaluated for disease severity and incidence 7, 14 and 21 days after the initial inoculation, using a rating scale modified from Bull et al., (2007). A rating of 0 was given for plants with no disease; 1, for plants with few lesions of < 3mm; 2, for plants with lesions > 3mm; 3, for plants with coalesced lesions; 4 for plants with many coalesced lesions < 30 of any leaf; 5, for plants with 50% or greater of any leaf diseased; 6, for plants with dead leaves.

In some experiments materials were also screened for the hypersensitive response as described below.

B. **Screen bacterial strains available in our collection for their ability to overcome resistance for bacterial leaf spot currently deployed.**

Bacterial suspensions of *X. campestris* pv. *vitians* strains prepared as described above were used to infiltrate 4 week old lettuce tissue or tissue from *L. serriola* accessions. For each experiment there were at least two replications per treatment with at least two infiltration sites per rep. Hypersensitive response was recorded for plant/pathogen combinations in which an incompatible reaction (cell collapse and death) occurred by 30 hours after infiltration. A compatible reaction was recorded for plant/pathogen combinations for which disease progressed and was visible only at 54 hour after infiltration. These experiments need to be repeated to confirm the results.

C. Collect and monitor strains of *X. campestris* pv. *vitians* isolated from diseased lettuce throughout California for ability to overcome resistance.

Drought conditions in California this year reduced the significance of bacterial leaf spot. Although several requests to growers were made, no reports or samples of bacterial leaf spot were reported from California fields. We therefore continued to refine our ability to identify strains with different genotypes. We use an approach that involves the sequencing of several different genes (multilocus sequencing) to identify various genetic genotypes of pathogens that differ for their ability to cause disease on various resistant cultivars. Previously four genes were used to define five distinct genotypes of *X. campestris* pv. *vitians*. During this funding cycle we sequenced an additional two genes. The *gap*1 and *lac*F genes from over 120 strains were sequenced and phylogenetic trees showing relationships among strains were generated using CLCbio Main Workbench.

RESULTS AND DISCUSSION

A. Provide BLS phenotype evaluations for Lettuce Breeding objectives.

We collaborate with the USDA/ARS lettuce breeding genotype. These collaborations resulted in CDFA SCBGP funding (2010-2013) developing baby leaf lettuce with enhanced resistance to *X. campestris* pv. *vitians* and we applied for additional funding this year. In this funding cycle bacterial leaf spot resistance was evaluated in germplasm resistant to Downy Mildew, Verticillium Wilt race 1, tipburn, leafminer, and dieback. In addition germplasm with improved shelf life was evaluated. Incomplete resistance from Batavia Reine des Glaces was fixed in populations that segregate for leaf type. These populations were recently released and published (Hayes et al., 2014). Likewise, complete resistance derived from La Brillante was used to develop a resistant iceberg type. This material has also been published and released (Simko, et al., 2014). Some *X. campestris* pv. *vitians* strains overcome resistance conferred by Little Gem,

La Brillante, and Pavane. Potential sources of resistance to some of these strains were identified in *L. serriola* accessions (see below). Additional objectives for bacterial leaf spot can be found in the USDA/ARS breeding report.

B. **Screen bacterial strains available in our collection for their ability to overcome resistance to bacterial leaf spot currently deployed.**

We previously described five distinct genotypes of *X. campestris* pv. *vitians* collected worldwide. We demonstrated that resistance conferred by Little Gem, La Brillante, and Pavane was effective against most, but not all strains evaluated. Our recent results show that 14 strains evaluated were not controlled by these cultivars. The 14 strains belong to two genotypes (Genotypes A and C). Infiltration of strains from Genotypes A and C into leaves of Little Gem (LG), Pavane (PV) or La Brillante (LaBr) result in compatible interactions (disease), whereas infiltration of strains from genotypes B, D, and E incite an HR or incompatible reaction in these cultivars (Figure 1). This is consistent with results from pathogenicity experiments for which the pathogens are spray inoculated onto the plants.

We also evaluated the interaction between various geneotypes of *X. campestris* pv. *vitians* and *Lactuca serriola* lines that were shown to be resistant to BS3127 (genotype A). We evaluated the HR in these lines using a representative strain from each genetic genotype. The data indicated that interactions between *L. serriola* and strains from genotype A were incompatible (expressed and HR) whereas interactions with strains from genotype C were compatible and resulted in disease (Table 1). This experiment was expanded to include all strains from Genotype A and C available. In both experiments, the reaction of the *L. serriola* lines to Genotype A strains was rapid cellular collapse and drying at the infiltration site. In many cases it appeared to be very similar to the reaction to *X. hortorum* pv. *hederae,* which was used as a control. *X. hortorum* pv. *hederae* is closely related to *X. campestris* pv. *vitians* but is not a pathogen of lettuce. The response (nonhost/pathogen) to this organism was rapid and more severe than the HR. Based on these and additional results seven of the nine initial *L. serriola* PI lines will move forward into the breeding program.

These data helped identify a new research goal. None of the cultivated lettuce or *L. serriola* lines tested to-date have shown complete resistance to strains from genotype C. Thus we will work to identify sources of resistance to genotype C strains. Genotype C strains may be important to California lettuce production because strains of this genotype were isolated in 2010 from King City and Santa Maria, Calif. To-date this genotype has not been isolated from other locations.

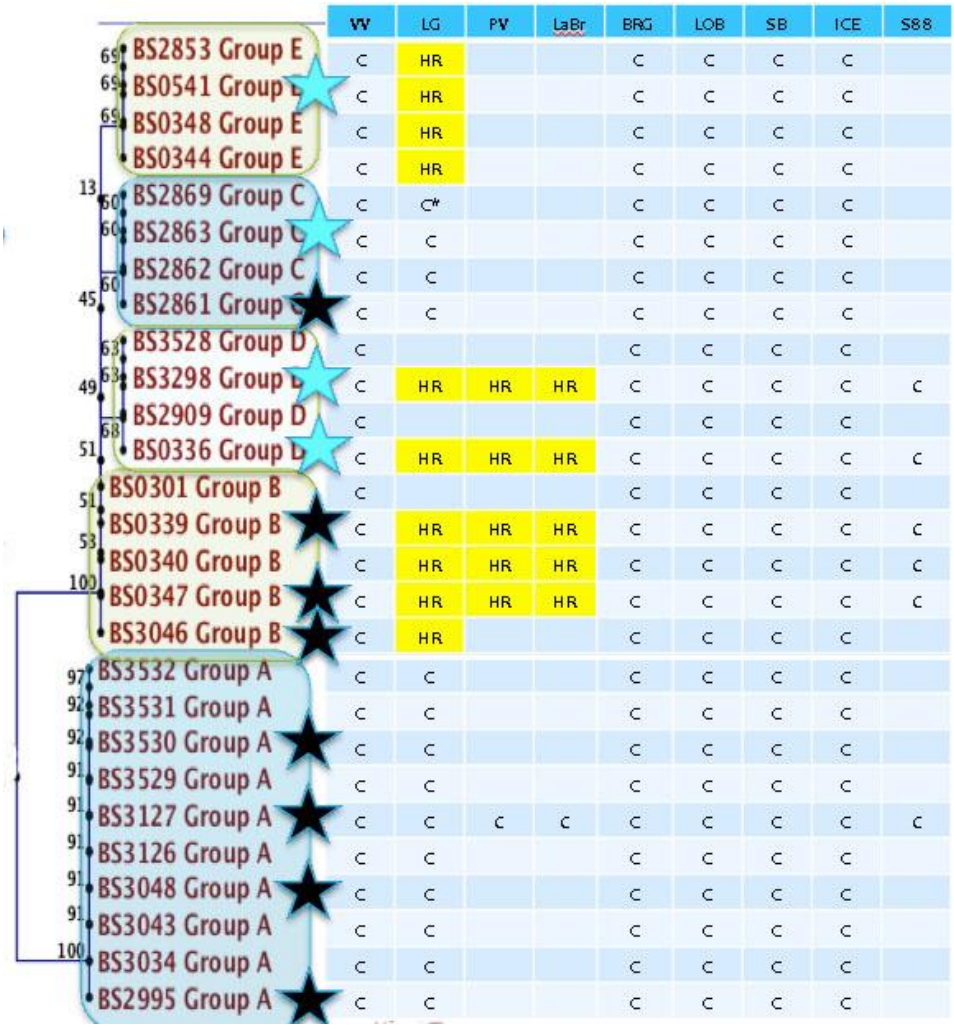

Figure 1. Compatible (disease) and incompatible (HR) reactions for individual strains on various cultivars of lettuce. Black stars indicate strains for which we have obtained complete genome sequences and blue stars are those we will sequence in the future. Vista Verde (VV), Little Gem (LG), Pavane (PV), La Brillante (LaBr), Batavia Reine des Glaces (BRG), Lobjoits (LOB), Salad Bowl (SB), Iceberg (ICE), Salinas 88 (S88). HR, indicates that cell collapse and drying occurred within 30 hours after inoculation at the site of infiltration. C, indicates that infiltrated region was wet and blackened by 54 hours after inoculation. Blank cells indicate combinations that have not been tested.

C. Collect and monitor strains of *X. campestris* pv. *vitians* isolated from diseased lettuce throughout California for ability to overcome resistance.

Our previous data indicate that we need to ensure that the cultivars we are deploying are resistant to the isolates of the pathogen present in lettuce fields in the region. Therefore we have genotyped over 120 strains of *X. campestris* pv. *vitians* and are evaluating the disease interactions between plant genotypes and pathogen diversity. The *gap-1* and *lacF* genes were sequenced for all the strains of *X. campestris* pv. *vitians* in our collection. The *lacF* gene did not reveal additional diversity beyond the five genotypes reported previously. The *gap*-1 gene did reveal additional diversity such that the 91 strains in genotype B were divided into two

genotypes with approximately equal numbers of strains. So far pathogenicity on specific cultivars or PI lines has been correlated with the genotype of the pathogen, thus we have been able to evaluate a few strains of each genotype on plant material we are testing. The data gathered this funding cycle prompts us to include representatives from each of the subgroups of genotype B when screening germplasm in the future. It is important to remember that there are at least six genetic genotypes of *X. campestris* pv. *vitians* to be considered when breeding for resistance.

ADDITIONAL INFORMATION TO REPORT

We recently published a specific PCR based detection system for the pathogen causing corky root of lettuce, *Sphingobium suberifaciens* (formerly *Rhizomonas suberifaciens*).

Bull, C.T., *Goldman, P H.*, and Martin, K.J. Novel primers and PCR protocols for the specific detection and quantification of *Sphingobium suberifaciens in situ*. Molecular and Cellular Probes http://dx.doi.org/10.1016/j.mcp.2014.03.001 2014.

Table 1. Hypersensitive response of *Lactuca serriola* PI lines to *Xanthomonas campestris* pv. *vitians* strains.

			Lactuca serriola lines						Controls	
Strain	Genetic Genotype	491104	491107	491108	491111	491114	491121	491122	Little Gem	Salinas 88
BS3107	Non-host control	HR	HR	HR	HR	HR	HR	HR	HR	HR
Buffer	Negative Control	NR								
BS3046	B	NI	C	C	C	C	C	C	HR	C
BS0347	B	C	C	C	C	C	C	C	HR	C
BS0340	B	C	C	C	C	C	C	C	HR	C
BS3270	B	C	C	C	C	C	C	C	HR	C
BS3127	A	HR	HR	HR	HR	HR	HR	HR	C	C
BS3126	A	HR	HR	HR	HR	HR	HR	HR	C	C
BS2995	A	HR	HR	HR	HR	HR	HR	HR	C	C
BS3048	A	HR	HR	HR	HR	HR	HR	HR	C	C
BS3529	A	HR	HR	NI	HR	HR	HR	HR	C	C
BS3530	A	HR	HR	HR	HR	HR	HR	HR	C	C
BS3043	A	HR	HR	HR	HR	HR	HR	HR	C	C
BS3034	A	HR	HR	HR	HR	HR	HR	HR	C	C
BS3531	A	HR	HR	HR	HR	HR	HR	HR	C	C
BS3532	A	HR	HR	HR	HR	HR	HR	HR	C	C
BS0344	E	C	C	C	C	C	C	C	HR	C
BS0348	E	C	NI	C	C	C	C	C	HR	C
BS0541	E	C	C	C	C	C	C	C	HR	C
BS2853	E	C	C	C	C	C	C	C	HR	C
BS2861	C	C	C	C	C	C	C	C	C	C
BS2862	C	C	C	C	C	C	C	C	C	C
BS2863	C	C	C	C	C	C	C	C	C	C
BS2869	C	C	C	C	C	C	C	C	C*	C
BS0336	D	C	C	C	C	C	C	C	HR	C
BS3298	D	C	C	C	C	C	C	C	HR	C

BS# represents bacterial strains culture number. All but BS3107 (*Xanthomonas hortorum* pv. *hederae*) are strains of *Xanthomonas campestris* pv. *vitians.*

HR, indicates that cell collapse and drying occurred within 30 hours after inoculation at the site of infiltration.

C, indicates that infiltrated region was wet and blackened by 54 hours after inoculation.

NI, indicates that this combination was not infiltrated.

This experiment needs to be repeated to confirm results.