Final Report submitted to the Pennsylvania Vegetable Marketing and Research Program
Pennsylvania Vegetable Growers Association

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Title: Identification of genetic resistance to tomato bacterial diseases in Pennsylvania

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Summary

The objective of this project was to test a collection of tomato accessions for resistance to the bacterial spot pathogen, *Xanthomonas perforans*. Resistant tomato accessions would be useful for tomato breeding efforts to improve resistance of cultivated tomato to bacterial spot disease. A Pennsylvania isolate of *X. perforans* was selected for the tests. A core group of seven tomato accessions was inoculated either by spraying with *X. perforans* bacterial suspensions or by dipping the aerial portions of plants into suspensions of *X. perforans*, and evaluating disease symptom progression over the course of one week. These tests indicated that six of the seven core accessions were moderately resistant to *X. perforans*, while one was more susceptible than the others. Quantitative bacterial growth in leaf tissues was also tested for three of the seven core accessions, including the one susceptible accession and two of the more resistant accessions. Quantitative bacterial growth is tested by infiltrating known concentrations of bacteria into the intercellular air pockets of tomato leaves and then measuring bacterial populations in the leaves over time after inoculation. The susceptible accession supported higher bacterial growth than one of the resistant accessions, as expected. However, the other resistant accession also supported high bacterial populations, like the susceptible accession. This pattern could be explained by two different mechanisms of resistance to *X. perforans*. An accession resistant to *X. perforans* when inoculated by dipping, spraying, or infiltration might have some innate immunity to *X. perforans* that takes effect as the bacteria enter into the plant leaves. In contrast, an accession resistant to *X. perforans* when inoculated by spraying but not when inoculated by infiltration, might have a resistance mechanism involving exclusion of *X. perforans* from the leaf interior in some way. The infiltration inoculation method bypasses the need for bacteria to enter the leaf on their own, which is the more natural mode of infection, and therefore bypasses certain types of plant disease resistance mechanisms. This project has provided an initial view of bacterial spot resistance among a core collection of tomato accessions. This project will continue in 2014

with screening of additional *S. pimpinellifolium* accessions as well as tomato breeding lines developed at Penn State for resistance to *X. perforans*.

Project Results

Tomato inoculations by spraying plants with bacteria (*Xanthomonas* perforans)

Xanthomonas perforans bacteria were removed from frozen stock vials and streaked on LB (Lysogeny Broth) media plates and grown at 28°C for 48 hours. Bacteria were then removed from the plates by scraping and suspended in 10 millimolar (mM) magnesium chloride ($MgCl_2$) solution. The bacterial concentration was adjusted to be approximately 100,000,000 bacteria per milliliter (ml) using a spectrophotometer. The spectrophotometer was set to measure absorbance at 600 nanometer (nm) wavelength, and the suspension was adjusted to an optical density at 600 nm (OD_{600nm}) of 0.1, which we empirically determined to represent 100,000,000 bacteria per ml by plating serial dilutions of the suspension and counting colonies. This is a standard procedure for creating a uniform bacterial inoculum for plant disease resistance tests.

Tomato plants were grown in pots in a greenhouse or growth chamber for 5 weeks prior to inoculation. Inoculations were performed in a greenhouse and plants were maintained in a high-humidity greenhouse for one week after inoculation. A disease severity scale of 0-100 was used to evaluate the visual percentage of severity, as shown in **Figure 1** below.

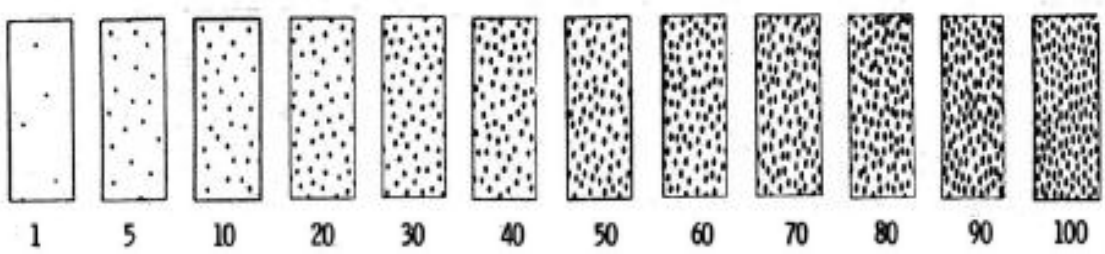

Figure 1. The disease severity scale used to evaluate the visual percentage of severity. Spots indicate bacterial disease lesion spots on leaves or on stems. Density of spots is translated into a disease severity score.

Tomato accessions PSLP 101, 121, 125, 127, 135, 136, and LA 1269 were tested by *X. perforans* bacterial spray inoculation as described above. At 7 days after inoculation, disease symptoms (disease response) were assessed using the scale shown in **Figure 1**. Two persons independently rated each plant. Symptoms on the leaves and on stems were recorded for each plant. **Figure 2** shows results for leaf symptoms, and **Figure 3** shows results for stem symptoms.

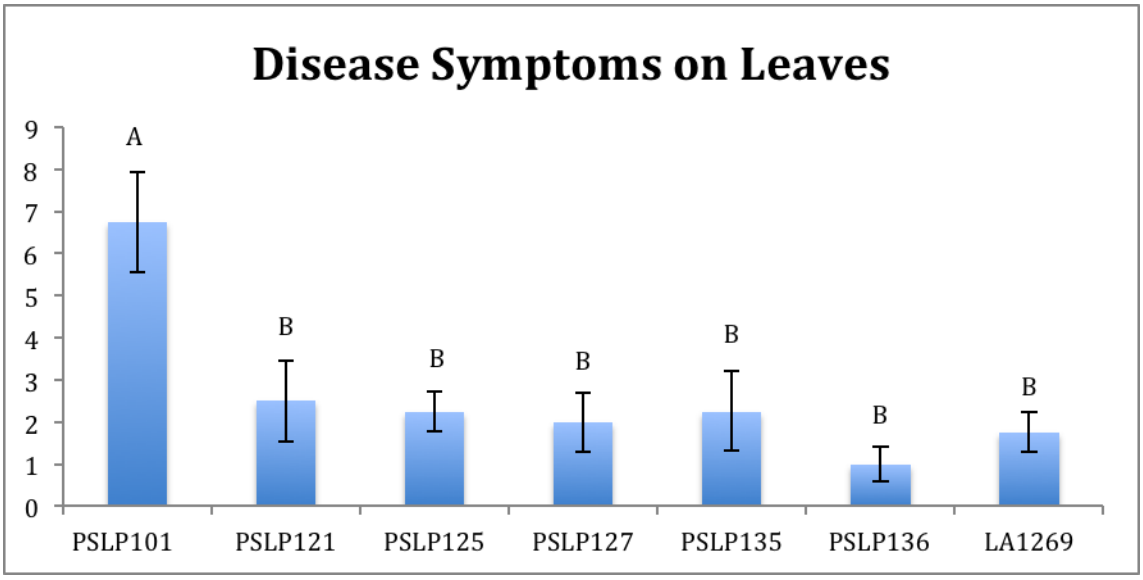

Figure 2. Disease symptom severity on tomato leaves at 7 days after inoculation with *X. perforans* by spraying. PSLP 101 had significantly more disease symptoms than the other six accessions according to Student's t-test. Bars with the same letter have no statistically significant difference. Y-axis shows disease severity score. Standard error bars are shown.

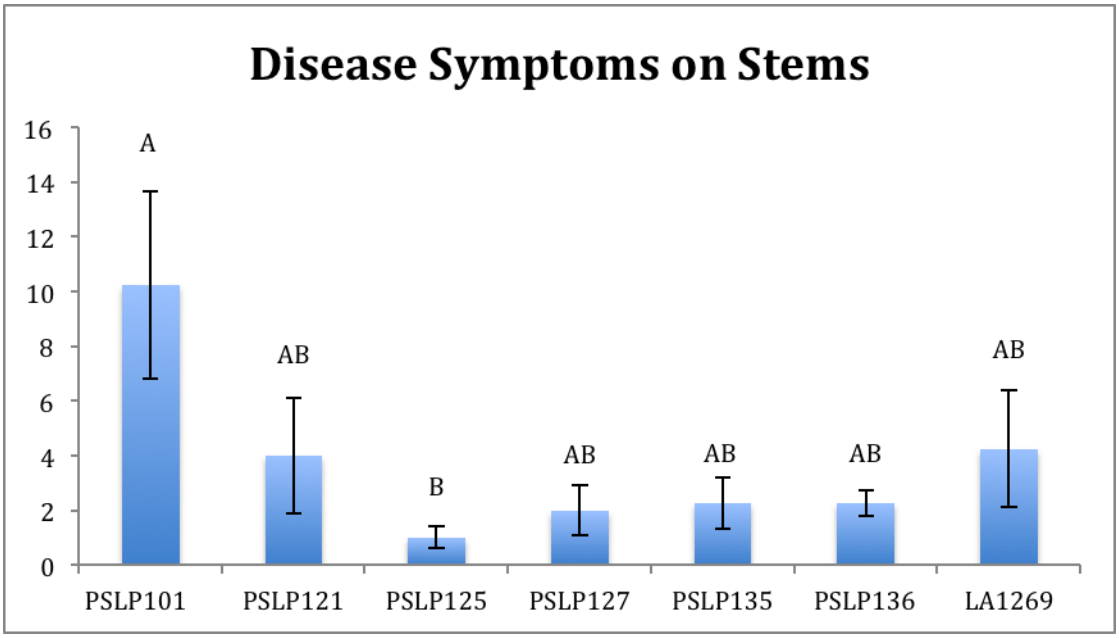

Figure 3. Disease symptom severity on tomato stems at 7 days after inoculation with *X. perforans* by spraying. PSLP 101 had significantly more disease symptoms than PSLP 125 according to Student's t-test. Bars with the same letter have no statistically significant difference. Y-axis shows disease severity score. Standard error bars are shown.

Overall, patterns of leaf and stem disease severity were consistent, with PSLP 101 having higher disease severity on both leaves and stems than any of the other genotypes tested. Based on these assays, PSLP 101 was selected as a "susceptible" genotype. In addition,

PSLP 125 and PSLP 136 were selected as "resistant" genotypes based on their very low levels of disease symptom development, with PSLP 125 having particularly low disease symptom development on stems and PSLP 136 having the least disease symptom development on leaves.

Quantitative disease resistance assays

Quantitative disease resistance assays were then performed with PSLP 101, 125, and 136. For this protocol, bacteria are introduced into the intercellular spaces and air pockets inside the leave through the stomata using a syringe without a needle. A relatively low concentration of bacteria is used compared to the spray inoculation method, usually about 1,000 - 10,000 bacteria per ml of inoculum. This method of inoculation provides a uniform concentration of bacteria within the tomato leaf tissue. Increases in bacterial population are then monitored by taking leaf samples, extracting them in water, and determining bacterial numbers by serial dilution plating of the extracts and counting of the resulting bacterial colonies on the plates. To be consistent, the sixth, seventh, and eighth leaves were selected for inoculation. Leaf number did not have any detectable effect on bacterial growth. Extracts were made at two, four and six days after inoculation. Bacterial populations at six days showed the greatest differences between tomato genotypes, and they are shown in **Figure 4**.

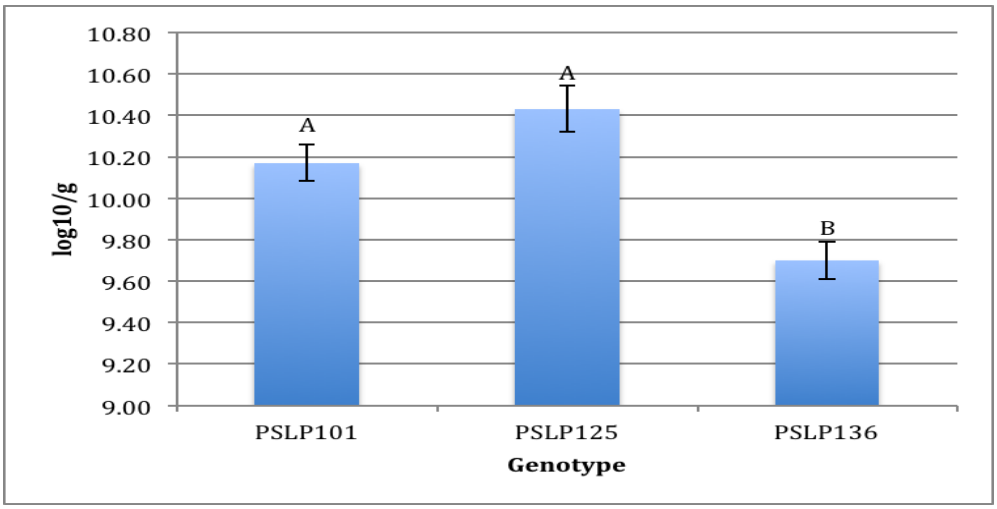

Figure 4. Quantitative bacterial populations in tomato leaves at 6 days after inoculation with *X. perforans* by syringe infiltration. Bacterial populations are shown using a logarithmic base 10 scale, which means that there are approximately 10 times as many bacteria in PSLP 125 leaves ad in PSLP136 leaves at this time point. Bacteria per gram of host leaf tissue are shown to allow comparisons between genotypes. PSLP 101 and PSLP 125 had significantly higher *X. perforans* populations at day 6 than PSLP 136 according to Student's t-test. Bars with the same letter have no statistically significant difference. Standard error bars are shown.

The results shown in **Figure 4** indicate that PSLP 101 supported higher *X. perforans* growth than did PSLP 136, as expected based on the less severe disease symptoms observed on PSLP 136 compared to PSLP 101 after spray-inoculation (**Figures 2 & 3**). However, the results with PSLP 125 were a bit surprising, since this genotype was expected to be more resistant, like

PSLP 136, based on the spray inoculation results. However, PSLP 125 supported relatively high growth of *X. perforans*. This result suggests that PSLP 125 and PSLP 136 might have different mechanisms of resisting *X. perforans*, and there is precedence for this type of resistance pattern in bacterial speck disease of tomato. PSLP 125 resistance could involve partial exclusion of the bacteria from the interior of the leaf, which they must enter in order to grow and cause disease symptoms. The leaf syringe infiltration procedure bypasses the need for natural bacterial ingress into leaves, which is part of the normal infection process. Therefore, PSLP 125 might be fairly resistant to natural infections, but not when syringe-infiltrated with *X. perforans*. PSLP 136 resistance appears to hold whether spray- or syringe-inoculated.

Conclusions

This project succeeded in identifying several tomato germplasm with varied levels of resistance to *X. perforans*. The project also allowed Drs. Foolad and McNellis to establish experimental systems to assay germplasm resistance to bacterial spot disease using a Pennsylvania isolate of *X. perforans* bacteria. However, the number of genotypes successfully screened for resistance to *X. perforans* was quite small. This was partially due to difficulties in initially developing the *X. perforans* inoculation system and losses of tomatoes in the greenhouse due to a pythium outbreak. However, we will continue this project to assess a wider range of germplasm, including a large collection of *S. pimpinellifolium* accessions and tomato breeding lines developed at Penn State, for resistance to *X. perforans*.