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Pennsylvania Vegetable Growers Association
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Title: Evaluating the status of copper resistance in bacterial populations in Pennsylvania tomato fields.

Principle Investigators:

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Introduction:

Bacterial spot, speck and canker are a constant challenge for tomato growers not just in Pennsylvania but in the mid-Atlantic and Northeast regions. Bacterial spot was once recognized as being caused by four races of *Xanthomonas campestris* pv. *vesicatoria* due to the variation within the pathogen population. More recently, these four races have now been reclassified into four new species named *X. vesicatoria*, *X. euvesicatoria*, *X. perforans* and *X. gardneri*. Bacterial speck is caused by *Pseudomonas syringae* pv. *tomato* and bacterial canker is caused by *Clavibacter michiganensis* subsp. *michiganensis*. Despite the implementation of strict sanitation programs starting with the use of heat-seed treatment and continuing through transplant and field production, outbreaks caused by these pathogens can still lead to extensive on-farm losses although losses tend to be highly variable between farms.

During the growing season, copper-based products are the primary tool used to reduce bacterial spread within and between plants. Copper is typically applied in a fixed form which lowers its solubility in water. Once applied to the plant surface, copper ions are slowly released when the plant surface becomes wet. When copper ions come in contact with a bacterial cell, they function to denature proteins thereby destroying enzymes necessary for the bacterial cell to function. Since copper is a protectant, once the bacteria enter the plant it is no longer exposed to the copper ions. The efficacy of coppers varies and is highly correlated with the amount of elemental copper is applied as well as how it is formulated. Finely ground copper products are more active than more coarsely ground products.

One increasing concern is the reduced efficacy of copper due to the development of resistance within the different tomato bacterial populations. In the U.S., bacterial spot resistance to fixed copper has been reported in Florida, Georgia, North Carolina, California, Tennessee, Oklahoma and Ohio while bacterial speck resistance has been reported in California and Virginia. Fortunately, copper resistance has not been reported with bacterial canker. Resistance develops due to selection pressure from frequent use of copper and is distributed through the movement of seed and transplants. In regions where copper resistance is a problem growers have had to reduce their reliance on copper-based fungicides and if used, tank mix it with mancozeb. Growers have also integrated the use of systemic acquired resistance inducing products such as Actigard as well as bacteriophage (a virus specific to the bacterial pathogen) into their management programs to help manage copper-resistant populations but losses continue to occur annually.

In Pennsylvania, it is not known if copper resistance within the bacterial populations affecting tomato is contributing to the increased difficulty growers are having managing these diseases. Therefore in

2015, we propose to conduct a survey of select growers fields to better understand the status of copper resistance in Pennsylvania and therefore facilitate more informed management decisions.

Methods and Results:

Unfortunately (or fortunately), 2015 turned out to be less favorable for bacterial spot on tomato across Pennsylvania than the 2014 growing season. In fact a bacterial spot product efficacy trial was unsuccessful despite multiple inoculation attempts and the use of supplemental overhead irrigation. Despite this, symptomatic tomato (and/or pepper) plants were collected from Centre, Clinton, Lehigh, Adams, Bucks, Berks and Schuylkill Counties for a total of 7 tomato and 4 pepper samples.

Symptomatic leaves from each sample were removed, surface sterilized, and a small piece of symptomatic tissue was macerated in a microcentrifuge tube with sterile distilled water. The sample was then plated on King's B (KB) selective media at a series of dilutions, incubated overnight at 86°F. Depending on the number of bacterial colonies that grew, a specific dilution was selected and again plated on KB media. Based on morphology, select colonies were transferred to new agar plates to obtain pure cultures. Pure cultures were grown over night in liquid LB broth and frozen for cyro-storage in 15% glycerol at -80C. This will enable us to keep the isolates for use in the long-term future.

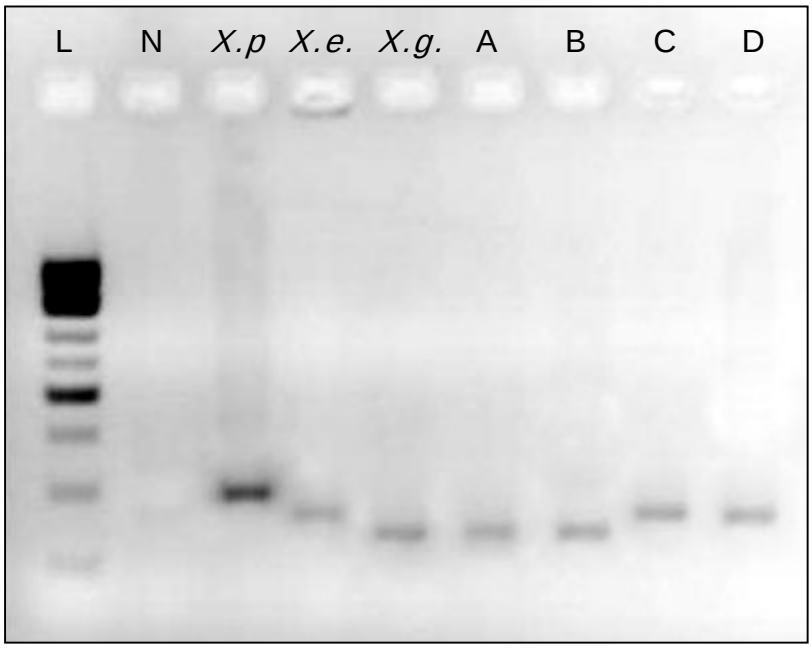

Fig 1. Multiplex PCR gel for the identification of three Xanthomonas species that cause bacterial spot on tomato and/or pepper. The column labels are as follows: L = 100bp ladder; N = water only negative control; Xp = *Xanthomonas perforans*; Xe = *X. euvesicatorai*; Xg = *X. gardneri* all are known reference isolates. Columns A to D are unknown samples. A and B = *X. perforans* from tomato; C and D = *X. gardneri* from pepper.

Bacterial isolates from the symptomatic leaves were identified using molecular techniques that use diagnostic primer sets to distinguish between the four different bacterial spot pathogens simultaneously. A separate set of primers are used for the identification of bacterial speck. Known pathogen isolates (reference isolates) are also included in for comparison to the unknown isolate. In 2015 not surprisingly, due to the cooler weather, bacterial speck caused by *Pseudomonas syringae* pv. *tomato* was confirmed as causing symptoms on three of the tomato samples. Bacterial spot caused by *Xanthomonas perforans* was confirmed on another three samples and *X. gardneri* was confirmed on two of the pepper samples. Based on conversations with vegetable pathologists in adjacent states, it is not surprising that we see both bacterial spot and speck especially considering that bacterial speck is more common to our north in New York and bacterial spot is more common to our south in Maryland. Due to the small sample size in 2015, it was decided to postpone the copper resistance screening until 2016 after more isolates have been collected.