Characterizing Pathogen Phenotypes and Diversity to Inform Management Decisions to control Bacterial Spot of Tomato in North Carolina

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Background

- A serious disease caused by at least 4 species of *Xanthomonas* including 4 races (Table 1).
- New races evolved even before the deployment of any resistant cultivar.
- There are no commercial resistant cultivars that confer resistance to bacterial spot of tomato.
- Management is based on cultural practices and chemicals such as copper, antibiotics (streptomycin) and plant activators.
- Failure of these chemicals and antibiotics for disease control have been reported from different regions of North Carolina.
- An understanding of the genetic diversity of strains comprising the population of *Xanathomonas* will aid in disease management and breeding against the bacterial spot pathogen.

Table 1. Species, races, and group of *Xanthomonas* causing bacterial spot on tomato.

Species	Group	Race
X. euvesicatoria	A	T1
X. vesicatoria	B	T2
X. perforans	C	T3, T4
X. gardneri	D	T2, T4 (?)

Objectives

- To characterize the bacterial spot strains of western North Carolina.
- To determine the proportion of field strains sensitive to copper and streptomycin.
- To determine the genetic diversity of the populations using rep-PCR.

Sample Collection

- Symptomatic leaf samples collected using a hierarchical sampling scheme from Western and Piedmont North Carolina for two years (Figure 1).
- Pathogens were isolated in the lab.

289 isolates collected from 27 fields across 8 counties over 2 years

Figure 1. Map of sampling. Red stars designate counties that are sampled for the bacterial spot pathogen of tomato.

Copper and Streptomycin Sensitivity

- The bacteria were assayed on Sucrose Peptone Agar (SPA) medium amended with different concentrations of copper (100ppm, 200 ppm, and 300 ppm) and streptomycin (20ppm, 50 ppm, and 100ppm) for 36-48 hours at 27 °C.
- Known resistant/susceptible strains were included as positive controls; non-amended media was used as a negative control.
- Strains capable to grow on the SPA media containing 200 ppm of $CuSO_4$ and 100 ppm of streptomycin were considered as copper and streptomycin resistant, respectively (Figure 2b).

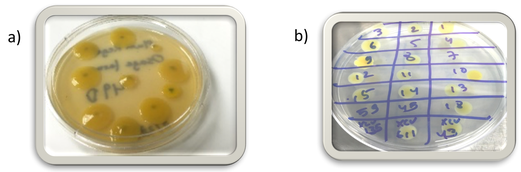

Figure 2. a) Isolation of bacteria from symptomatic leaves b) Growth of bacteria in 200 ppm copper sulfate amended SPA medium.

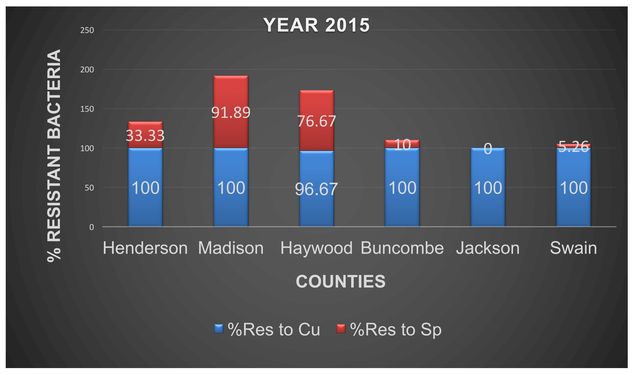

Figure 3. Summary of copper and streptomycin sensitivity test of bacterial isolates collected in year 2015.

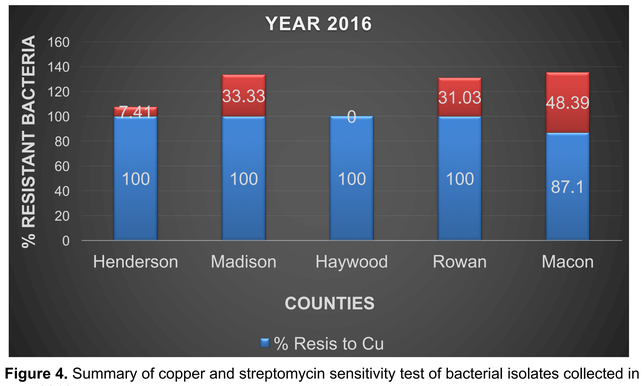

Figure 4. Summary of copper and streptomycin sensitivity test of bacterial isolates collected in year 2016.

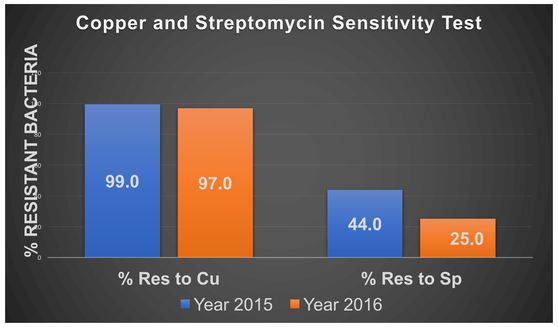

Figure 5. Percentage of bacterial strains resistant to copper and streptomycin in 2015 and 2016.

Genetic Diversity Analysis

- Strains were assessed for genetic diversity using BOX-PCR (BOXA1R: 5'-CTACGGCAAGGCGACGCTGACG-3').
- The fingerprint profiles of the strains were scored visually : '0' for the absence of the band and '1' for the presence of the band to generate a binary matrix, which was used for cluster analysis.

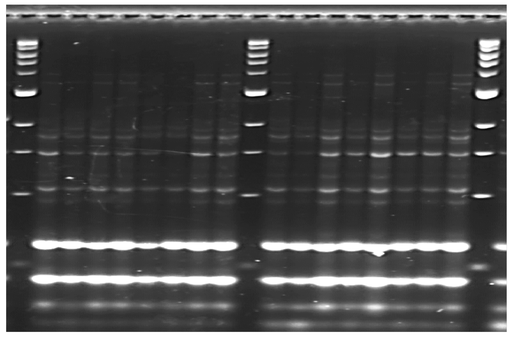

Figure 6. BOX-PCR fingerprints of strains of *Xanthomonas spp.* of tomato from NC.

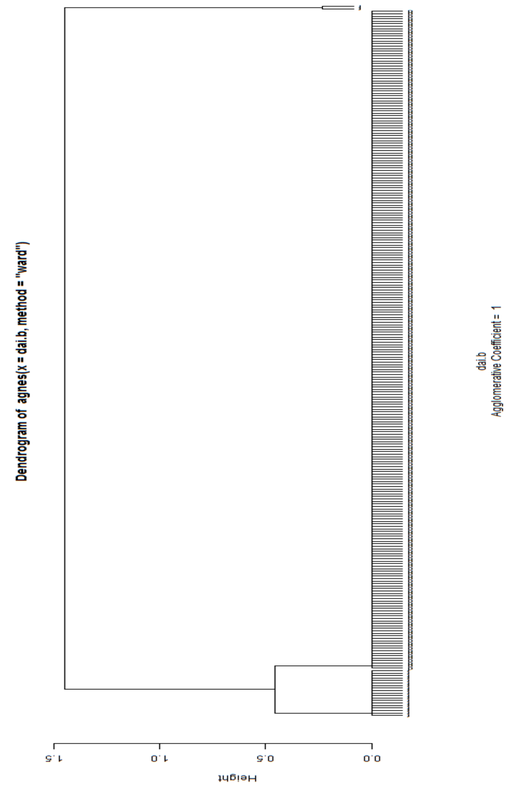

Figure 7. Hierarchical agglomerative nesting cluster analysis of strains of *Xanthomonas spp.* based on BOX-PCR fingerprints.

Recommendations

Growers

- Use Copper only if needed to control other sensitive pathogens and then in combination with other measures.
- Use streptomycin in the transplant production phase.

Industry

- Durable host resistance is desperately needed.

Conclusions

- Widespread existence of copper resistance and a modest level of streptomycin resistance.
- Bacterial isolates from NC are genetically similar, despite different seed and transplant sources. This raises questions about the origination, spread and persistence of the pathogen. They were all *X. perforans.*
- This project will further classify the bacteria for race.
- In turn, these data will be helpful for developing effective disease management strategies and to optimize our current breeding program to develop bacterial spot resistance in tomato cultivars.

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