INTRODUCTION

Bacterial speck of tomato, incited by *Pseudomonas syringae pv. tomato* (Pst) is an economically important disease of tomato (*Lycopersicon esculentum* Mill.) in the western production region of North Carolina. The bacterial pathogen is commonly introduced on contaminated seed (Goode and Sasser, 1980) or overwintered host debris (McCarter et al. 1983). Pst can incite leaf lesions, defoliation, fruit lesions and, in cases of sufficient disease pressure, yield loss of marketable fruit. A low incidence of contaminated seed can lead to a high incidence of disease in the field as a result of epiphytic multiplication and distribution on the phyllosphere of the crop plant.

Practical management of bacterial speck is a challenge in commercial production fields due to limited efficacy of current disease management strategies. For example, copper applications on tomato crops at the Mountain Horticultural Crops Research Station in Fletcher NC did not reduce disease incidence, suggesting some Pst strains may be copper resistant. Likewise, host resistance has not proven durable. Virulent strains of Pst lacking *avrPto* were documented in natural populations (Lawton and MacNeill 1986) even prior to deployment of identified resistance conferred by the Pto gene.

OBJECTIVE

The objectives of this study were to characterize Pst populations in tomato fields of Western North Carolina, to determine the proportion of field strains sensitive to streptomycin and copper, to determine the genetic diversity of the populations using rep-PCR, and to initiate disease management practices to limit losses.

MATERIALS AND METHODS

Fourteen farms were surveyed for incidence of bacterial speck symptoms in 1999 and 13 farms in 2000. Foliar disease incidence and fruit speck incidence was recorded for each site. Twenty symptomatic leaves were collected in a systematic manner from each field for isolation of Pst strains. Strains were isolated on Vogel-Bonner tartrate medium. Isolated strains were characterized for streptomycin and copper sensitivity and genomic fingerprint profiles using BOX-PCR. Strains known to be sensitive and resistant to streptomycin and copper were included as controls. Up to 40 representative strains were evaluated for HR on tobacco and pathogenicity on tomato seedlings.

Twelve seed, transplant and field spray treatments were evaluated for tomato bacterial speck control at the Mountain Horticultural Crops Research Station, Fletcher, NC in 2000. Treatments were arranged in a randomized complete block design with four replications and as a factorial
experiment (2 x 2 x 3) with two levels of seed treatment, 2 levels of antibiotic use during transplant growth, and three levels of foliar applied treatments in the production field. The test was repeated in 2001 but with fewer treatments (not a complete factorial). Seed were inoculated under vacuum with *Pst*. Treatments included: 40 min seed soak in 20% (v/v) Clorox prior to sowing or not treated; three weekly streptomycin (200 ppm) sprays on transplants starting at first true leaf stage or no antibiotic; and weekly field sprays with standard fungicides plus either none, Kocide 2000 1.5 lb (1-15th appl) or Actigard 50WG 0.67 oz. NC recommendations for fresh market tomato production were used. Five-week-old seedlings were transplanted on to raised beds previously fumigated with 150 lb/A methyl bromide (67%) + chloropicrin (33%) and covered with 1.25 mil black polyethylene. Plants were spaced 2 ft apart in rows 5 ft apart. Plots were single rows, four plants each, separated at each end by 12 ft and on each side by 10 ft from adjacent plots. Field spray treatments were applied weekly at 2X conc utilizing a Stihl model 400 back-pack mistblower operated to provide complete coverage. Spray volume began at 15 gpa and was increased by 10 gpa weekly to a maximum 75 gpa. Disease ratings (modified Horsfall-Barratt) were made weekly and vine-ripe fruit were harvested weekly.

**RESULTS**

Bacterial speck symptoms were observed in 10 of 14 fields surveyed in 1999 and all 13 fields surveyed in 2000. Foliar disease incidence ranged from 0% to 25% and fruit speck incidence ranged from 0% to 20%. A total of 200 isolates were successfully obtained from 20 fields (n = 1 to 20 per field). All strains verified as *Pst* were copper resistant and streptomycin sensitive. Representative strains induced HR when infiltrated into tobacco leaves and incited speck symptoms on young tomato seedlings when assessed for pathogenicity. All strains characterized by BOX-PCR had similar genomic fingerprint profiles (Figure 1) despite different seed and transplant sources in the 20 fields and surveyed over two years.

![Bacterial speck strains](image1.png)

**Figure 1:** BOX-PCR Fingerprints of *P. syringae pv. tomato*
Highlighted results of the trials designed to evaluate management strategies are included in Tables 1 and 2. Fruit infection was increased by Kocide applications in 2000 and not affected by Kocide in 2001. Actigard decreased bacterial speck incidence and severity on the foliage and fruit both years compared to use of standard fungicides alone and standard fungicides plus Kocide. Clorox seed treatment, streptomycin transplant treatment and Actigard field treatments were additive in their influence on reducing foliar and fruit symptoms of bacterial speck in 2000. In 2001 this additive effect was not observed.

Table 1: Highlights of treatments when used alone or in combination on incidence of bacterial speck symptoms and marketable fruit yield in 2000.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Seed</th>
<th>Transplant</th>
<th>Field spray (bacterial)</th>
<th>Foliar % LAS</th>
<th>Total no. spots</th>
<th>Percent fruit</th>
<th>Marketable fruit T/A</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>48.3 a&lt;sup&gt;4&lt;/sup&gt;</td>
<td>462 bc</td>
<td>46 bc</td>
<td>22.6 cd</td>
<td>2000</td>
</tr>
<tr>
<td>Clorox</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>41.8 ab</td>
<td>326 ef</td>
<td>44 c</td>
<td>22.3 cd</td>
<td>2000</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Kocide</td>
<td>41.8 ab</td>
<td>344 cde</td>
<td>39 cd</td>
<td>23.0 cd</td>
<td>2000</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Actigard</td>
<td>2.1 d</td>
<td>271 fef</td>
<td>32 de</td>
<td>26.0 bc</td>
<td>2000</td>
</tr>
<tr>
<td>Clorox</td>
<td>Strep</td>
<td>None</td>
<td>Actigard</td>
<td>1.4 d</td>
<td>138 h</td>
<td>22 f</td>
<td>35.0 a</td>
<td>2000</td>
</tr>
</tbody>
</table>

<sup>1</sup>% LAS = percent leaf area showing bacterial speck symptoms (leaf spots and marginal necrosis).

<sup>2</sup> Total number of bacterial speck spots counted on fruit per four-plant plot and percent of fruit with one or more spots.

<sup>3</sup> Marketable yield includes US grades No. 1, 2 and 3 and excludes fruit with bacterial speck symptoms.

<sup>4</sup> Means with the same letter are not significantly different (P=0.05) by Waller-Duncan k-ratio (k=100) test.

Table 2: Highlights of treatments when used alone or in combination on incidence of bacterial speck symptoms and marketable fruit yield in 2001.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Seed</th>
<th>Transplant</th>
<th>Field spray (bacterial)</th>
<th>Foliar % LAS&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Percent fruit infection&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Marketable fruit T/A&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>41.6 a</td>
<td>55.5 ab</td>
<td>7.2 cd</td>
</tr>
<tr>
<td>Clorox</td>
<td>None</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>Strep</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Kocide</td>
<td>22.0 bc</td>
<td>43.1 b</td>
<td>9.9 bc</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Actigard</td>
<td>3.5 d</td>
<td>24.4 c</td>
<td>18.8 a</td>
</tr>
<tr>
<td>Clorox</td>
<td>Strep</td>
<td>None</td>
<td>Actigard</td>
<td>4.2 d</td>
<td>28.3 c</td>
<td>12.5 b</td>
</tr>
</tbody>
</table>

<sup>1</sup>% LAS = percent leaf area showing bacterial speck symptoms (leaf spots and marginal necrosis).

<sup>2</sup> Percent of fruit with one or more spots.

<sup>3</sup> Marketable yield includes US grades No. 1, 2 and 3 and excludes fruit with bacterial speck symptoms.

<sup>4</sup> Means with the same letter are not significantly different (P=0.05) by Waller-Duncan k-ratio (k=100) test.
CONCLUSIONS
Bacterial speck is a widespread problem in the tomato production region of western North Carolina. In some fields, high levels of fruit speck incidence were responsible for substantial reductions in marketable fruit.

Copper resistant Pst strains were prevalent in all fields consistent with many grower observations that copper sprays were not effective to limit bacterial speck incidence. Copper resistance in Pst populations has been reported recently in VA and Canada (Alexander, 1999; Cuppels and Elmhirst, 1999). Our field trials also demonstrated that copper applications can increase bacterial speck incidence. Growers will need to rely on other methods to limit bacterial speck. Our field trials demonstrated that the integrated use of seed treatments, transplant treatments, and field use of Actigard can reduce losses due to bacterial speck. Actigard has proven useful to limit field incidence of bacterial speck and spot in tomato production (Louws et al. 2001).

The genetic uniformity of the Pst population was also of interest. The strains were isolated from fields grown to different tomato varieties and originating from different transplant production greenhouses. Lack of genetic diversity among Pst populations from diverse production regions limited our ability to understand possible sources of inoculum.

This study highlights that bacterial speck is a widespread problem in the Western production region of North Carolina. Isolates obtained over two years were consistently tolerant of copper and sensitive to streptomycin. Reduction of disease risk may best be achieved through the combined use of seed treatments, transplant sprays, and field use of Actigard. Bacterial canker continues to occur in this production region also and additional work is required to learn if Actigard will offer sufficient control or if copper will continue to be used by growers in the region to manage the canker epidemics.

REFERENCES