

2006 O.T.R.I. EXECUTIVE SUMMARY REPORT

PROJECT TITLE: Ecological/epidemiological studies of the highly virulent Group C and D forms of the bacterial spot pathogen

RESEARCHER: Dr. Diane A. Cuppels, Agriculture and Agri-Food Canada, London

INTRODUCTION: Bacterial spot continues to be a problem for tomato and pepper growers in Ontario. Current copper-based control programs have had limited success. The bacterial spot strains isolated from Ontario fields over the past few years have been more aggressive than those found in earlier years. Our studies have shown that most of these strains belong to Group D, once considered a rare form of this pathogen. Group C forms also have been isolated. Little is known about the ecology of the Group C and D strains. We have been examining their ability to overwinter on buried debris and to survive epiphytically on weeds or non-host crops. Also, we continue to refine our methods of detecting these bacteria.

PROJECT OBJECTIVES:

I. Determine a) **the effect of greenhouse copper sprays** on the ability of **PCR assays** to detect bacterial spot on transplant seedlings and b) **the effectiveness of these sprays in reducing pathogen populations on seedlings.** **II.** Continue studies to determine the **long-term survival** of these pathogens **on buried plant debris** and their **ability to colonize weeds adjoining fields used for tomato propagation.**

METHODOLOGY:

I. Effect of copper sprays . In vitro. A cell suspension of the bacterial spot causing xanthomonad (BSX) strain DC93-1 was added to Kocide suspensions, ranging in concentration from 2.75 mg/ml to 27.5 ng/ml. After 30 min at 25 C, the bacteria were removed by centrifugation and the number of viable cells in each suspension was determined using CKTM-Bravo growth medium. DNA was extracted from each suspension using the Sigma REDExtract-N-Amp Plant PCR kit and PCR was performed using the DNA extracts as templates and the bacterial spot-specific AAFC-BSX1/2 as primers. The experiment was performed three times. **In planta.** Tomato seedlings (cv. N1082) were grown in 288-well plug trays using a standard protocol. At 4 weeks, two trays were sprayed with BSX strain DC93-1; one tray was left as a control. At five weeks, one infected tray was sprayed with Kocide 101 at 5.5g/L. Two days later, foliage was assayed for the number of viable cells (cfu)/g of tissue (using CKTM-Bravo); DNA was extracted from the rest of each suspension as described above or with Sigma GenElute Bacterial Genomic DNA kit and then subjected to PCR. The experiment was repeated five times and there were three reps/treatment/experiment.

II. Development of new DNA probes. PCR products, obtained using 1) the BSX1/2 primers and cloned DNA from Group D strain DC00T7A and 2) the Florida primers (Jeff Jones' lab) JJ19/22 and Group C strain DC00T15A DNA, were labeled using the Roche DIG Labeling Mix. Cell suspensions were made of each bacterial strain to be tested against the new probes and then spotted onto a Roche nylon membrane in quadruplicate. The blots were subjected to hybridization with the new probes and processed. The results were visualized using X-ray film. **Overwintering experiment.** Tomato plant debris, collected from AAFC experimental plots that had been infected with Group B or D strains, was buried in nylon bags at a depth of 10 cm on the AAFC-London farm in mid-November 2004. The bags were recovered in April 2006 and sampled for the presence of the bacterial spot groups using AAFC DNA probes and CKTM-Bravo medium plus various antibiotics. **Screening weeds for BSX.** In mid July 2006, specimens (foliage and roots) of various weed species, were collected from a corn field that had been used for tomato production in 2005 and had been severely infected with bacterial spot A 10-g sample from each specimen was washed in sterile water and the bacteria were collected from the wash water by centrifugation. The pellet was re-suspended in 2 ml of sterile water; the suspension then was serially-diluted and plated on CKTM + Bravo agar. Colony blots were made and probed with the new bacterial spot DNA probes. Pathogenicity tests were done on select probe (+) colonies.

RESULTS:

I. Effect of copper sprays . In vitro. Copper interfered with PCR assays using bacterial cell suspensions as template but only when it was at a very high concentration (2.75 mg/ml). PCR assays using copper-sprayed plants as templates were inhibited when the Sigma REDExtract-N-Amp Plant PCR kit was used but not when the Sigma GenElute Bacterial Genomic DNA kit was used to isolate DNA. Our *in vitro* assays (with bacterial cell suspensions) indicated that copper-treated cells, which could not be recovered on a bacterial growth medium, gave a positive PCR at copper concentrations of 275 or 27.5 µg/ml. Whether the positive PCR was due to residual DNA from dead cells or due to some of the copper-exposed bacteria going into a viable but nonculturable condition remains to be determined. **In planta.** Although not yet showing symptoms 9 days after inoculation, the control plants (no Kocide) harbored 3 million to 177 million bacteria/g of tissue. Kocide reduced the bacterial spot populations substantially but it did not eliminate them. Numbers on Kocide-treated plants ranged from 20,000-2 cells/g of foliar tissue. In one experiment, plants also were sampled 9 days after the Kocide spray; populations on the Kocide-sprayed plants increased from 50 to 1500 cells/g but never reached the populations of the control, indicating that even just one copper spray can have a significant impact on pathogen populations.

II. Development of new DNA probes. FLA-C was generated using PCR primers designed by J. Jones' lab to specifically detect Group C or Race T3 strains and TA425-D was generated using AAFC PCR primers BSX1/2 with cloned Group D strain DC00T7A DNA as the template. These probes were tested against 4 Group A BSX strains, 3 Group B BSX strains, 7 Group C BSX strains, 11 Group D BSX strains and 46 non-pathogenic xanthomonad strains isolated from weeds, healthy tomato plants or wheat roots. FLA-C hybridized exclusively to the 7 known Group C strains and to none of the other strains in the screen. TA425-D hybridized to all known Group D strains plus 8 strains from the other BSX groups A, B and C; it did not hybridize to any of the non-pathogenic xanthomonads in the screen. Thus, when used together, probes TA425-D and FLA-C should be able to detect all Group C and D strains present in a sample.

Overwintering. In our previous experiments (2004/2005), representatives of Group B, C and D strains survived the winter well on buried tomato/pepper plant debris from the previous season. In this year's study, no BSX bacteria were detected in unearthed tomato/pepper plant debris that had been infected with Group B or D bacteria and buried in November 2004. Thus this experiment suggests that the Group B and D bacterial spot pathogens will not survive more than a few months on buried debris in Ontario field soil.

Presence on weeds. Plant tissue was collected from 19 species of weeds (44 samples) in or at the edges of an Ontario corn field that had been used for tomato production in 2005 and had been heavily infected with bacterial spot. Using the FLA-C and TA425-D DNA probes, we found no Group C BSX bacteria but TA425D (+) populations were present at low levels (100-24,000 cells/g) in 7 of the 44 samples (4 species: curled dock, goldenrod, quackgrass and wild buckwheat). In 2 of the 3 quackgrass samples, numbers exceeded 10,000/g of tissue. When tested on tomato plants, one of the quackgrass isolates was pathogenic and identified as bacterial spot; we are continuing to test isolates from quackgrass for pathogenicity. No BSX populations were found on the tomato relatives hairy and black nightshade, nor on volunteer soybean plants from the edge of the field.

SUMMARY

- Kocide interference with PCR assays on sprayed plug plants was remedied by using a different DNA isolation kit. Copper-treated cells which could not be recovered on a growth medium, can give a positive PCR. Whether these cells have entered a viable but nonculturable condition remains to be determined.
- Although Kocide does not eliminate the bacterial spot pathogen from tomato leaf surfaces, even just one spray has a significant impact on pathogen populations.
- The bacterial spot pathogen, shown to survive one winter on buried plant debris, could not be recovered after a second winter of being buried on the AAFC-London research farm.
- New DNA probes for bacterial spot were developed to screen weeds for the presence of bacterial spot. We found no Group C strains but confirmed bacterial spot bacteria were present in a quackgrass sample, which may be a happenstance occurrence. We hope to continue with this weed survey in 2007.