

2005 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

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INTRODUCTION: Bacterial spot continues to be a problem for tomato and pepper growers in Ontario. Current copper-based control programs have had only limited success. The bacterial spot strains isolated from Ontario fields over the past five years have been more aggressive than those found in earlier years. Our studies have shown that most of these strains belong to Group D, a once rare and unusual form of the bacterial spot pathogen which infects both tomatoes and peppers; the other isolates we have are members of Group C (Race T3), likewise a group not previously found in Ontario. Little is known about the epidemiology and epiphytic fitness of these bacteria.

PROJECT OBJECTIVES: **I)** Develop the PCR-based tools needed to perform ecological/epidemiological studies of the Group C and D forms of the bacterial spot pathogen, including a procedure for detecting and quantifying bacterial spot populations on transplant seedlings. **2005 Goal:** Refine our method for extracting bacterial spot DNA from seedling samples to increase the sensitivity/reliability of conventional and quantitative PCR assays. **II)** Characterization of Ontario bacterial spot strains (focussing on Groups C and D) with regard to overwintering and survival on volunteer tomato plants, wheat roots or weeds.

METHODOLOGY:

I) Comparison of different procedures for extracting bacterial spot DNA from leaf samples. Five 10-g leaf samples from tomato plug seedlings were collected and washed in sterile water. Various concentrations of BSX (bacterial spot-causing xanthomonads) were added to each of the wash water samples. The wash water samples then were centrifuged and the pellets were re-suspended in water; each suspension was divided into four 0.5- ml aliquots. One was used to determine the viable bacterial cell count and the other three aliquots were subjected to DNA extraction using the Qiagen DNeasy Plant Mini kit, the Sigma GenElute Bacterial Genomic DNA kit, or the Sigma REDExtract-N-Amp Plant PCR kit. Samples of healthy transplant seedlings from a commercial greenhouse also were used in this series of experiments. **Conventional PCR.** DNA extracts of the samples described above were used as templates in conventional PCR assays. using the bacterial spot-specific AAFC-BSX1/2 primers and the xanthomonad-specific RST primers. **Real-time quantitative PCR assay.** The Roche LightCycler Instrument was used to quantify bacterial spot DNA recovery using the various DNA kits described above. Standard curves were prepared using kit-purified genomic DNA from the Ontario bacterial spot strain DC93-1.

II) Overwintering experiment. Tomato/pepper plant debris, collected from AAFC experimental plots that had been infected with Group A, B, C or D strains, was buried in nylon bags at a depth of 10 cm on the AAFC-London farm in mid-November 2004. Before being buried, each leaf debris bag was spiked with a culture of the respective antibiotic-resistant Group strain used in our 2004 field studies. The bags were recovered in April 2005 and sampled for the presence of the bacterial spot groups using the AAFC-BSX probe KK1750 and selective bacterial growth media. **Screening volunteer plants, wheat roots or weeds for BSX.** In late June and July 2005, specimens of various weed species, volunteer tomato plants and wheat roots were collected from fields that had severe bacterial spot in 2004: 1 carrot field, 2 wheat fields, 1 corn field and 1 sugar beet field. 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: 0.5 ml was serially-diluted and plated on CKTM + Bravo agar and the other 1.5 ml was frozen at -70 C. Colony blots were made and probed with the bacterial spot DNA probe KK1750.

RESULTS:

I Refine our method for extracting bacterial spot DNA from seedling samples. Screening plug greenhouses for the presence of BSX bacteria requires a procedure capable handling relatively large samples of tomato or pepper seedling leaves. If we are to use PCR-based methods in the screening, we must develop a protocol that is able to effectively remove the large quantities of PCR inhibitors that usually are present in such large samples. We tested several different DNA extraction kits for their ability to remove these inhibitors and recover BSX DNA. The Sigma REDExtract-N-Amp Plant PCR kit was the most effective, improving BSX DNA recovery over our previous method by 5 to 10-fold and allowing us to detect 1000 bacteria/g of leaves (approximately a ten-fold improvement).

II. Characterization of Ontario bacterial spot strains with regard to overwintering and survival on weeds or volunteer plants. Overwintering. The overwintering results for 2005 resembled those of 2004. Group C and D strains were able to survive the winter well, numbers exceeding 100,000 bacteria/g, on buried infected tomato or pepper leaf debris. The numbers on buried fruit were 10-fold lower. Group B also was detected on tomato and pepper leaf debris but the populations were 1000-fold lower. Group A strain did not survive the 2004-2005 winter on any of the buried material. **Presence on weeds, wheat roots or volunteer tomato plants.** Plant tissue from volunteer tomatoes, wheat and twenty-three species of weeds (92 samples total) were collected in or at the edges of five Ontario fields (2 wheat, 1 carrot, 1 sugar beet and 1 corn) that had been used for tomato production in 2004. The bacterial spot pathogen was detected in 7 of the 14 wheat root samples tested, three samples harboring populations exceeding 10,000 cells/g of tissue. Only 5 of 15 volunteer tomato plants tested supported the pathogen. The pathogen was found on a number of weeds (27 of 63 samples), mostly in very small numbers. However, populations exceeded 10,000/g on 3 wild carrot samples and the one green foxtail and one catnip sample collected. It appears that the bacterial spot pathogen can survive as an epiphytic population on a number of plant species, many of which are unrelated to tomato. Black nightshade, a tomato relative, had relatively small populations of this pathogen.

SUMMARY AND CONCLUSIONS:

Previously, using artificially-infested samples of plug tomato leaves, we were able to detect the equivalent of approximately 1000 bacterial cells/PCR reaction (or 10,000 cells/g leaf tissue). However, we found that the PCR results were not consistent across all sample replicates, probably due to the presence of high concentrations of PCR inhibitors in some of these samples. In the present set of experiments we tested several different DNA extraction kits and have identified a kit that provides more consistent results with conventional PCR, even when the bacterial population is low and the number of PCR inhibitors is high. The percent recovery of bacterial spot DNA was 10 to 5-fold higher than that obtained with the kit we had been using previously, allowing us to detect 100 cells/PCR reaction.

Our two-year overwintering study indicated that BSX Group C and D can survive the winter well on buried tomato and pepper leaf and fruit debris. Group A strains were not good survivors, not being recovered from any of the samples in 2005. Group B strains were recovered in low numbers from tomato and pepper leaf debris only. We also have in progress a long-term survival study comparing Groups B and D. In the spring of 2006, 2007 and 2008, we will retrieve several replicate samples buried on the AAFC-London farm and test for the presence of the pathogens.

Specimens of various weed species (63 samples), volunteer tomato plants (15 samples) and wheat roots (14 samples) were collected from five fields (carrot, wheat-2, corn and sugar beet) that had severe bacterial spot in 2004. The bacterial spot pathogen was detected in 7 of the wheat root samples. These results confirmed those obtained by Kentucky researchers in 1946 (Diachun and Valteau, *Phytopathology* 6: 277-280). Only 5 volunteer tomato plants, all isolated from a corn field, supported the pathogen. The pathogen was found on less than half of the weed samples collected. Numbers were very low except for 1 green foxtail, 1 catnip, and 3 wild carrot samples. These results are preliminary and the survey will be repeated next year. We currently are performing an extensive physiological characterization of twenty strains randomly isolated from the wild carrot sample.