

## 2004 O.P.V.G. 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

### PROJECT OBJECTIVES:

- 1) 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 in the greenhouses.
- 2) To determine the epiphytic fitness of the Group C/D strains (in comparison with Groups A and B)(presence and persistence on plug plants, greenhouse equipment, etc; overwintering ability, survival on weeds, etc). Worldwide, no survival studies have been done on the Group C/D strains.

### METHODOLOGY:

**Assaying tomato seedlings from plug greenhouses for the presence of bacterial spot.** In early May and early June, a total of 64 plug plant samples were collected from 32 different sources (4 early-maturing and 4 later-maturing varieties). Detached leaves (15 g) from each of these samples were washed in sterile water and the bacteria were collected from the wash water by centrifugation. An aliquot of the pellet was used to perform a viable bacterial count using our bacterial spot-specific probe KK1750. DNA was extracted from the remainder of the pellet using a modification of Qiagen's Plant Dneasy kit. Conventional PCR was performed in our lab with the AAFC BSX primers and with the Univ. of Florida's RST primers; a sample of the DNA extract also was sent to M. Sabourin at the Univ. of Guelph's Pest Diagnostic Lab. After the plug plants had gone into grower fields, one plug plant source from each of the varieties was assayed twice (two weeks apart) and in duplicate(a total of 16 samples).

**Growth of bacterial spot Groups A, B, C and D on field tomato and pepper plants.** In early June, four tomato (cv 9553) field plots (six rows/plot; 20 plants/row) were planted on the AAFC-London research farm; three rows of each plot were sprayed throughout the season with Kocide DF-Bravo 500 F according to OMAF Recommendations. Likewise, four pepper (cv. Inferno) field plots (six rows/plot; 20 plants/row) were planted; three rows of each plot received Kocide DF sprays. Three weeks after planting, each plot was sprayed with one of the following pathogens: a Group A, B, C or D strain marked with antibiotic resistance (inocula were  $\sim 5 \times 10^8$  cells/ml). Bacterial populations were followed for 56 days. Fruit lesion number and yield per plant were determined 13 (pepper) or 15 (tomato) weeks after planting.

**Overwintering experiment.** In October 2003, tomato and pepper plant debris was collected from AAFC experimental plots that had been infected with Group A, B, C or D strains. Nylon bags each were filled with 30-g samples of leaf and stem debris or 190-g samples of tomato fruit. The bags were buried at a depth of 10 cm in a horizontal position in mid-November 2003. Before being buried, each leaf debris bag was spiked with a culture of the respective antibiotic-resistant Group strain used in the 2003 field studies. The bags were recovered in April 2004 and the contents sampled for the presence of the various bacterial spot Groups using PCR and selective bacterial growth media containing the various antibiotics.

## RESULTS

### I. Develop and implement a PCR-based procedure for detecting and quantifying bacterial spot populations on transplant seedlings in the greenhouses:

In the spring of 2004 we worked in cooperation with H.J. Heinz Company of Canada Ltd, Kraft Canada Inc and the Pest Diagnostic Lab at the University of Guelph to screen randomly-collected samples of transplant seedlings from the plug greenhouses for the presence of the bacterial spot pathogen using our PCR-based diagnostic assay. We extracted DNA from the 64 samples (32 sources) that the processors

delivered to our lab. We performed conventional PCR using the DNA extracts and then sent a portion of the extracts to the Pest Diagnostic lab. The Pest Diagnostic Lab also used our BSX PCR primers to screen for bacterial spot. No bacterial spot was detected in the 64 samples using the AAFC-BSX primers; the same results were obtained by the Pest Diagnostic Lab. When we used the RST primers (which amplifies xanthomonad DNA and is not exclusive to the bacterial spot pathogen), 6 of the 64 samples were positive. BSX bacteria were detected using the BSX DNA probe KK1750 in 4 of the samples; however, the number of bacteria present in these samples was low, ranging from 6 to 5000/g of tissue. Using the LightCycler and quantitative PCR we determined that recovery of bacterial DNA from artificially-infested greenhouse plug plants using our extraction method averaged 2%. Therefore, to be detectable by PCR, a sample would have to have at least 30,000 to 50,000 bacteria/g of tissue. Thus, our inability to detect the pathogen by PCR in these samples was to be expected. We also tested plants from 4 of the 32 sources after they had been planted in grower fields (two samplings, 2 weeks apart)(16 samples). Bacterial spot was detectable both by PCR and by the DNA probe assays in samples from all 4 sources. Typically, the second sampling had significantly higher numbers of the pathogen than the first sampling. Random sampling of bacterial strains from these fields indicated that they were Group D strains. The PCR and DNA probe results were not consistent across all the field sample replicates; we found that this was due to the presence of high concentrations of PCR inhibitors in some of the field samples.

**II. To determine the epiphytic fitness of the Group C/D strains of the bacterial spot pathogen:**

**Growth and disease development on field tomatoes and peppers.** In our second year of comparing Group A, B, C and D strains of the bacterial spot pathogen on tomato and pepper field plants, the results were similar to those obtained previously. Groups B, C and D grew well on tomatoes while Groups A and D grew well on peppers; the populations were slightly less than those recorded in 2003. With the exception of Group C-infected tomato plants, Kocide-Bravo more effectively curtailed populations in 2004 than in 2003. In both years, pepper fruit infection was rare. With the exception of Group C-infected plants, the number of tomato fruit lesions was significantly less in 2004 than in 2003, probably due to differences in weather conditions. Although Kocide-Bravo effectively reduced the number of tomato fruit lesions for all Groups in 2003, only the Group C and D-infected plants had fewer lesions than the unsprayed plants in 2004. The sprays significantly increased the yield of spot-free fruit in both years. Although the Group D form grew as aggressively as the Group B form on tomato plants, it caused fewer fruit lesions than Group B in both years. The results with the Group C form were inconclusive; additional experiments are merited.

**Overwintering.** Our overwintering studies indicated that Group B, C and D strains can survive the winter well on buried leaf and fruit debris. Interestingly, in late August 2004, we found Group D strains on volunteer tomato plants growing on the AAFC-London farm; these bacteria were not the tagged strains that we had introduced in 2003 and 2004, indicating that they may have come from introduced plug seedlings.

**CONCLUSIONS**

Using our AAFC-BSX PCR primers, we and the Pest Diagnostic Lab at Guelph did not find the bacterial spot pathogen in any of the 64 samples (32 sources) of plug plants assayed. However, low numbers of this pathogen were found in 4 of the samples using the AAFC BSX DNA probe assay, a more sensitive but also more time-consuming and labourious detection method. The pathogen was detected (PCR and probe) on field plants originating from 4 of the same plug plant sources we had assayed previously. Future work will be directed at improving the sensitivity of the our PCR assay with symptomless plug plants and field plants.

The Group A and D (but not B) forms of the bacterial spot pathogen grew well on field pepper plants in both test years; the Group C form is not able to infect pepper plants. Kocide more effectively curtailed bacterial spot populations in 2004 than in 2003, probably due to weather differences.. Although the Group D form (currently the most common form in Ontario) grew as well as the Group B form (prevalent in the mid 1990s) on field tomatoes, it produced fewer tomato fruit lesions in both test years. Preliminary results indicate that all three groups, B, C and D, are able to overwinter on buried tomato and pepper plant debris in Ontario.