New Disease Reports

First report of bacterial spot (*Xanthomonas cucurbitae*) of pumpkin in Ontario, Canada

C.L. Trueman [1]*, E. Roddy [2] and P.H. Goodwin [3]

[1] *Ridgetown Campus, University of Guelph, 120 Main Street East, Ridgetown, ON, N0P 2C0, Canada*; [2] *Ontario Ministry of Agriculture and Food, 120 Main Street East, Ridgetown, ON, N0P 2C0, Canada*; [3] *School of Environmental Sciences, University of Guelph, Guelph, ON, N1G 2W1, Canada*

*E-mail: ctrueman@uoguelph.ca

Received: *15 May 2014.* ***Published:*** *18 Aug 2014.* ***Keywords:*** *Cucurbita pepo, gyrase subunit B, lesion, soft rot, cucurbits, bacterial disease*

In August 2012, leaves of pumpkin (*Cucurbita pepo* cvs. Gladiator, Aladdin, Apollo, and Super Hero) in Kent County, Ontario, Canada were observed with 1-4 mm irregular-shaped light brown to tan lesions, often with a chlorotic halo. Mature fruit had 2-4 mm light brown to tan sunken lesions with dark borders (Fig. 1), and later developed severe soft rot. Approximately 35 ha were affected with more than 50% of foliage and 60% of fruit damaged. Isolations were made from fruit using the method described by Cuppels *et al*. (1990) but with tryptic soy agar. More than 95% of colonies isolated were opaque, light to bright yellow, glistening, circular, and flat. Isolates were gram-negative.

Substrate utilisation profiles (Biolog, Hayward, CA) of Ontario isolates CT12PT1A and CT12PT3 were compared with those described for *Xanthomonas* DNA homology groups (Vauterin *et al*. 1995). Utilisation patterns of the 95 substrates in the BIOLOG assay for CT12PT3 and CT12PT1A had 96.8% and 94.7% identity with members of Group 8 (*X. cucurbitae*), and both had 95.8% identity with at least one of the three *X. cucurbitae* isolates reported by Dutta *et al*. (2013) (Dutta, pers. communication). The 16S DNA sequences of CT12PT3 and CT12PT1A were obtained with primers designed by the Pest Diagnostic Clinic, University of Guelph (5'GCYTAACACATGCAAGTCGA-3' and 5'-GTGTGTACAAGNCCCGGGAA-3'). A BLAST search of the GenBank database revealed that both sequences (GenBank Accession Nos. KJ817203 and KJ817204) had 100% nt identity to the 16S DNA sequences of *Xanthomonas dyei* (NR_104949), *Xanthomonas pisi* (AB680442), *Xanthononas vesicatoria* (AY288080 and AF123088) and *Xanthononas cucurbitae* (AB680438).

In addition, 928 bp of the gyrase subunit B gene (*gyrB*) was sequenced with primers gyrB-F and gyrB-R (Hamza *et al*., 2012). Both Ontario isolates (KJ817205 and KJ817206) had 100% nt identity with *gyrB* of *X. cucurbitae* (HM569161.1 and HM569162.1). The next closest matches were *Xanthomonas axonopodis* pv. *citrumelo* (CP002914.1) and *Xanthomonas campestris* pv. *raphani* (CP002789.1), with 94% nt identity. A neighbour-joining tree from a ClustalW multiple sequence alignment of the overlapping regions of these *gyrB* sequences and 14 other *Xanthomonas* species showed that sequences from both Ontario isolates clustered only with HM569161 and HM569162 with high bootstrapping values (Fig. 2). These results strongly suggest the isolates are *X. cucurbitae*.

To confirm pathogenicity of the isolates, a bacterial suspension (1 x 10^7 cfu/ml) of each isolate was applied to the leaves of four pumpkin plants (cv. Howden) using a hand-held mist sprayer. Plants were covered for 24h under a translucent plastic box and maintained under artificial light with 16h day length at 24-28°C. Small lesions with slight chlorotic haloes were observed on all inoculated plants 10 days post inoculation (Fig. 3). No symptoms were observed in the water control. Bacteria were isolated from lesions as previously described. The *gyrB* sequences of these isolates had 100% nt identity to the original isolates, and the BIOLOG substrate utilisation tests showed 100% and 98.9% similarity to CT12PT3 and CT12PT1A respectively. The presence of bacterial spot caused by *X. cucurbitae* poses a new threat to pumpkin and squash production in Ontario.

References

Cuppels DA, Moore RA, Morris VL, 1990. Construction and use of a nonradioactive DNA hybridization probe for detection of *Pseudomonas syringae* pv. tomato on tomato plants. *Applied and Environmental Microbiology* **56**, 1743-1749.

Dutta B, Gitaitis RD, Lewis KJ, Langston DB, 2013. A new report of *Xanthomonas cucurbitae* causing bacterial leaf spot of watermelon in Georgia, USA. *Plant Disease* **97**, 556. http://dx.doi.org/10.1094/PDIS-10-12-0908-PDN

Hamza AA, Robene-Soustrade I, Jouen E, Lefeuvre P, Chiroleu F, Fisher-Le Saux M, Gagnevin L, Pruvost O, 2012. MultiLocus sequence analysis and amplified fragment length polymorphism-based characterization of xanthomonads associated with bacterial spot of tomato and pepper and their relatedness to *Xanthomonas* species. *Systematic and Applied Microbiology* **35**, 183-190. http://dx.doi.org/10.1016/j.syapm.2011.12.005

Vauterin L, Hoste B, Kersters K, Swings J, 1995. Reclassification of *Xanthomonas*. *International Journal of Systematic Bacteriology* **45**, 472-489. http://dx.doi.org/10.1099/00207713-45-3-472

Figure 1

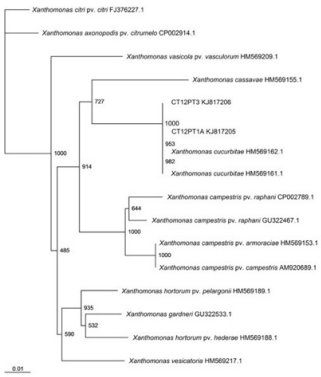

Figure 2

Figure 3

To cite this report: Trueman CL, Roddy E, Goodwin PH, 2014. First report of bacterial spot (*Xanthomonas cucurbitae*) of pumpkin in Ontario, Canada. *New Disease Reports* **30**, 8. http://dx.doi.org/10.5197/j.2044-0588.2014.030.008

This report was published on-line at www.ndrs.org.uk where high quality versions of the figures can be found.