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Subject: **Diagnosis of a new liberibacter species in tomato and capsicum**

The purpose of this report is to provide a summary of the process followed to diagnose the disease of tomatoes and capsicum reported in the Auckland area in 2008.

Summary of diagnostic process:

- The first glasshouse grown tomato samples were submitted by a grower in South Auckland on 25/01/08. The grower reported symptoms including spiky chlorotic apical growth, general mottling of the leaves, rugose curling leaves, bushy chlorotic head growth and general stunting of plants. Symptoms varied in severity and percentage affected between cultivars. The grower reported a high influx of tomato potato psyllid during November 2007 and he considered that the symptoms correlated with TPP numbers.
- IDC-PHEL's Virology & PEQ team tested the plants using PCR primers for geminiviruses (includes *Tomato yellow leaf curl virus*), *Cucumber mosaic virus*, *Tomato spotted wilt virus*, *Tobacco mosaic virus*, *Tomato chlorosis virus*, *Tomato infectious chlorosis virus*, Pospiviridae (includes *Potato spindle tuber viroid*), tombusviruses (includes *Tomato bushy stunt virus*), *Beet pseudo yellows virus*, ilarviruses, *Tobacco rattle virus*, potexviruses (includes *Pepino mosaic virus*), and phytoplasmas (four times using real-time and conventional PCR with a range of universal phytoplasma primers). All tests were negative. The plants were also tested using a generic double stranded RNA extraction gel, electron microscopy, and herbaceous indicators; again, all tests were negative.
- IDC-PHEL's Mycology & Bacteriology team could not detect any culturable pathogenic fungi or bacteria. The Entomology team confirmed the presence of whiteflies and tomato potato psyllid (*Bactericera cockerelli*).
- A second tomato grower reported the presence of similar tomato symptoms in late February 2008 to AsureQuality Plant Pest Lab. Plant samples requiring virus testing were forwarded to IDC-PHEL. Symptoms at the second site were described by the grower as narrowing and twisting of the leaves, rapid head decline and interveinal chlorosis.
- In both production facilities, symptoms appeared in small, random patches, radiating outwards in a roughly circular pattern rather than along the hydroponics rows, as would be expected if mechanical transmission were occurring.
- Due to the similarity of symptoms with those reported as psyllid yellows overseas, and the absence of a positive result for other possible causal organism, a tentative diagnosis of 'psyllid yellows' was made. 'Psyllid yellows' is caused by toxin that the immatures produce when they feed. Adults do not cause damage. Symptoms include upward curling of leaflets nearest the stem on the top of the plant. As the disease establishes itself, these symptoms become more evident. Plant yellowing is initially found on the leaf edges. Severe infection shows overall yellowing with enlarged nodes, development of clusters of small leaves in the auxiliary buds that appear rosetted, internodes shortened and the plant eventually becomes dwarfed.
- On 4/4/08, a second grower in South Auckland reported similar symptoms appearing in their adjacent capsicum crops. Plant growth was stunted and pale, followed by interveinal chlorosis, leaf cupping and curling, and shortened internodes, resulting in tip necrosis and plant death. Capsicums are not known to get 'psyllid yellows'.

- IDC-PHEL contracted HortResearch to examine symptomatic plants by transmission electron microscopy on thin sections of the affected plants. On 18/4/08, HortResearch reported that the tomato material contained a phytoplasma- or bacterium-like organism (BLO). They were also seen in the phloem of capsicum but not in sufficient numbers to make a positive diagnosis.
- A range of universal and specific 16S rRNA PCR primers were used in different combinations. Sequence analysis of the 16S rRNA gene is widely used to study the phylogeny of prokaryotes. Some of these primers also amplify chloroplast DNA. One of the primers combinations produced a fragment in both healthy and symptomatic samples as well as an additional band in only the symptomatic samples. The PCR product that was present in only the symptomatic samples and not the healthy sample, was cloned and sequenced. The resulting sequence received by IDC-PHEL on 8/5/08 covers about two-thirds of the 16S rRNA gene and has 97% sequence identity to the 16S rRNA gene of '*Candidatus* Liberibacter asiaticus' – the bacterium that causes huanglongbing in citrus (also known as citrus greening).
- On 1/5/08, a third grower in the Auckland area submitted 2 symptomatic cherry tomato plants to IDC-PHEL. Both tested positive by PCR as described above.
- Subsequently the remainder of the 16S rRNA gene has been sequenced. This has shown that unique signature sequence regions present in the citrus-infecting liberibacter species are not present in the tomato/capsicum liberibacter. To further compare the tomato/capsicum liberibacter to the known liberibacter species, two additional fragments of the genome (the 16S/23S rRNA spacer region and *rpl*KAJL-*rpo*BC operon) were amplified and sequenced. The sequence from the tomato/capsicum liberibacter shared 70-80% sequence identity with the citrus-infecting liberibacter species.
- A 16S rRNA phylogenetic tree was constructed using neighbour joining, maximum likelihood, and Bayesian inference. All trees showed that the organism is clearly a member of the genus '*Ca.* Liberibacter' but is distinct from the currently described species and strains. Phylogenetic analysis of the 16S/23S rRNA spacer region and the *rpl*KAJL-*rpo*BC operon confirmed this conclusion.
- A specific test has been developed for the detection of the tomato/capsicum liberibacter. The primer pair detected the liberibacter in all symptomatic tomato and capsicum plants (approximately 42 samples to date) from the 3 affected properties. No fragment was amplified from healthy plants or from the three species of liberibacter infecting citrus (the Asian, African, and American species).
- During these experiments, a variety of controls have been incorporated including:
  - Positive controls (infected material);
  - Negative controls (both water ["blank"] controls and healthy material grown from seed in quarantine); and
  - Internal PCR controls (separate reactions to verify the PCR-competence of the samples).

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