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SHORT RESEARCH NOTES

Candidatus Phytoplasma australiense is associated with pumpkin yellow leaf curl disease in Queensland, Western Australia and the Northern Territory

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Abstract. Pumpkin plants (*Cucurbita maxima* and *C. moschata*) with pumpkin yellow leaf curl (PYLC) disease were observed at production fields in Queensland, Western Australia and the Northern Territory. Diseased samples were positive for a phytoplasma indistinguishable from *Candidatus* Phytoplasma australiense, the phytoplasma associated with papaya dieback and strawberry lethal yellows. This is the first time *Candidatus* Phytoplasma australiense has been detected in pumpkin.

Phytoplasmas are phloem-limited plant pathogens which are associated with numerous plant diseases worldwide (Seemüller *et al*. 1998). In Australia, the most commonly detected phytoplasmas are tomato big bud (TBB) and *Candidatus* Phytoplasma australiense (Davis *et al*. 1997, 2003; Schneider *et al*. 1999; Padovan and Gibb 2001). Both these phytoplasmas are associated with diseases of papaya, strawberry and grapevine (Padovan *et al*. 1996; Gibb *et al*. 1996; Padovan *et al*. 2000). Worldwide, phytoplasmas are associated with numerous yellows diseases such as the coconut lethal yellows disease (Mpunami *et al*. 1999). Although records of yellows diseases in pumpkin are uncommon, one such record is in C. moschata cv. Kent (Jap) in Kununurra in 1997. The cause of the disease at the time was undetermined (Conde, unpublished records).

In 2000 and 2001, pumpkin plants with yellow, curled leaves and stunted growth were collected from the Queensland production areas in Gatton (two samples), Bundaberg (one sample) and Stanthorpe (one sample). The samples from the Gatton area (longitude 152; latitude –27) were from two separate farms within 1 km of Lockyer Creek and 7 km of each other. Neither papaya nor strawberry plants were present in this area but symptomatic cottonbush (*Gomphocarpus* sp.) was common in the area. The sample collected at Stanthorpe (longitude 151; latitude –28) was from pumpkin sown near a strawberry runner production area at Applethorpe Research Station; no papaya is grown in this district. The Bundaberg sample (longitude 152; latitude –24) was from a block at Bundaberg Research Station where a papaya plant, symptomatic for dieback, was also present at about 200 m from the single symptomatic pumpkin; commercial strawberries and backyard papaya were present within 5 km of this field. In 2003, pumpkins with the same symptoms were collected from Kununurra in Western Australia (two samples) and Katherine in the Northern Territory (four samples). The samples from Kununurra (longitude 128; latitude –15) were from a property that is adjacent to papaya production areas. Two samples from Katherine (longitude 132; latitude –14) were from two separate farms where papaya was grown until 2000. The other two Katherine samples were collected from farms where papaya is not grown.

The reference tomato big bud phytoplasma was extracted from periwinkle (*Catharanthus roseus*) plants maintained at Charles Darwin University (Darwin) and the reference *Candidatus* P. australiense was extracted from papaya dieback (PDB) diseased samples collected from Redlands (Queensland). These phytoplasmas were selected as reference strains because they are the most commonly detected phytoplasmas in Australia.

Total DNA was extracted from plant material according to Doyle and Doyle (1990). Pellets were resuspended

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in 50 µL of sterile distilled water and then stored at −20°C. Samples were screened using the polymerase chain reaction (PCR) with the 'universal' primer pair P1 (fP1; Deng and Hiruki 1991) and P7 (rP7; Schneider *et al*. 1995), which are specific for the 16S rRNA gene and adjacent spacer region of phytoplasmas. One microlitre of DNA was added to each PCR tube and amplification conditions were 95°C/1 min; 55°C/1 min; 72°C/1.5 min for 35 cycles.

Deoxyribonucleic acid samples were also screened by PCR using primers (fMLO1 and rMLO1) that amplify the phytoplasma elongation factor (*tuf*) gene of the aster yellows group including *Candidatus* P. australiense (Schneider *et al*. 1997). The *tuf* gene is not amplified from the TBB phytoplasma using this primer pair (Schneider *et al*. 1997). The PCR was prepared according to Schneider *et al*. (1997) and amplification conditions for the *tuf* primer pair were 95°C/1 min; 50°C/1 min; 72°C/1.5 min for 35 cycles.

Products amplified using the fP1 and rP7 primers were digested with the restriction enzymes *Alu*I and *Rsa*I while those amplified using the *tuf* gene primers were digested with the restriction enzymes *Hpa*II and *Hin*dIII. Ten microlitre digestion reactions contained buffer (Promega, Sydney), 1 U enzyme (Promega), 5 µL of PCR product and SDW. Digestions were performed according to the manufacturer's specifications and bands were separated on a 12% polyacrylamide gel. RFLP gels were stained with ethidium bromide and bands observed under a UV transilluminator.

The symptomatic pumpkin samples collected from Gatton, Bundaberg and Stanthorpe were positive for phytoplasma using the universal test (data not shown). One of the two samples from Kununurra and one of the four samples from Katherine were positive for phytoplasma using the universal test (data not shown). The amplified products had the same banding pattern after digestion with *Alu*I and *Rsa*I (data not shown). The RFLP banding patterns of the diseased pumpkin samples were distinguishable from the TBB phytoplasma and indistinguishable from the banding pattern previously reported for *Candidatus* Phytoplasma australiense (Davis *et al*. 1997; Gibb *et al*. 1998; Liefting *et al*. 1998).

The PCR primers specific for the *tuf* gene (fMLO1 and rMLO1) amplified a product from the diseased pumpkin samples collected in Gatton, Bundaberg and Stanthorpe (data not shown). The sample from Kununurra that was positive for the 16S rRNA gene PCR primers also gave a band after amplification with the *tuf* primers (data not shown). Four samples collected from Katherine tested positive for the *tuf* gene although two signals were very faint and so they were not subjected to RFLP analysis. RFLP analysis of the *tuf* gene PCR products showed that the diseased pumpkin samples all gave the same banding pattern as the PDB reference phytoplasma (Fig. 1).

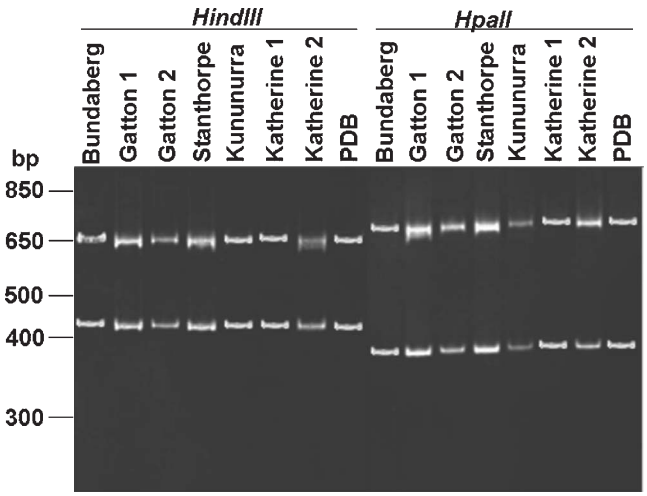

Fig. 1. RFLP banding pattern of the *tuf* gene amplified from pumpkin yellow leaf curl samples collected from Bundaberg (QLD), Gatton (QLD), Stanthorpe (QLD), Kununurra (WA) and Katherine (NT). The reference sample of *Candidatus* Phytoplasma australiense is papaya dieback (PDB).

Our results show that the phytoplasma *Candidatus* Phytoplasma australiense is associated with pumpkin yellow leaf curl (PYLC) disease in Australia. *Candidatus* Phytoplasma australiense has previously been reported as the causal agent of the important diseases, papaya dieback (Gibb *et al*. 1996), Australian grapevine yellows (Padovan *et al*. 1996), strawberry lethal yellows and strawberry green petal (Padovan *et al*. 2000). This is the first record of *Candidatus* Phytoplasma australiense in pumpkin. The finding is significant because pumpkin crops are often grown in close proximity to papaya crops in Queensland and WA where dieback can cause significant losses (Gibb *et al*. 1998; K. Gibb, unpublished data). Pumpkin crops can also be grown in close proximity to strawberry fruit and runner production crops. In Katherine, there were commercial papaya plantations operating up until 2000 and dieback has been recorded from papaya there in the past (Padovan *et al*. 2000). The identification of pumpkin as a host for *Candidatus* Phytoplasma australiense is an important step towards unravelling the phytoplasma transmission cycle which may involve a number of plant host species. Future management strategies will need to accommodate this host range information, but the critical missing piece is the identity of the insect vector.

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