New Disease Reports

First report of a carlavirus infecting plants in the *Fabaceae* in Australia

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In 2016, severe pod distortion and leaf mottling in green beans (Phaseolus vulgaris) for the fresh market (Figs. 1-2; isolate Q5288) occurred in the Fassifern production area in south Queensland, Australia. Disease incidence in crops was 60-100%, resulting in losses of up to \$AUD 400,000. A sample of soybean (Glycine max cv. Zam-1) displaying similar leaf symptoms (Fig. 3; isolate Q5294) was collected from a crop, about 50 km from the infected bean crops. Disease incidence in this crop was 5-10%. Both isolates were transmitted by manual inoculation and by Bemisia tabaci (MEAM1) from infected bean plants to uninfected soybean and bean. All test plants developed symptoms similar to the original samples. The inoculated plants tested negative for potyvirus using an ImmunoStrip test (Agdia, USA) and a group-specific RT-PCR (Langeveld et al., 1991; Gibbs & Mackenzie, 1997). Flexuous virions 600-700 nm in length (Fig. 4) were observed in the inoculated plants The virion morphology is consistent with carlaviruses and the samples tested positive in DAS-ELISA with antibodies (DSMZ, Germany) for the carlavirus, Cowpea mild mottle virus (CPMMV).

The virus has since been detected in Australia's major winter bean production area, some 1000 km distant from the original detection site. In 2019, the carlavirus was also identified from Bundaberg in south Queensland where disease incidence exceeded 50% with significant production losses from deformed, unmarketable pods. The detection of the virus in regions producing 80% of Australia's fresh green beans and the high susceptibility of major commercial varieties has prompted new research on virus epidemiology and management.

Amplicons covering part of the coat protein gene through to the 3' end of the genome were obtained by RT-PCR amplification using the oligo-dT primer Poty 1 (Gibbs & Mackenzie, 1997) as the reverse primer for both isolates forward Carla7190F and the primer (5'-GGNYTNGGNGTICCIACIGARCAYGT-3'; designed to detect a Q5288 of carlaviruses) for and CPMMV7277F range (5'-GATTCHAGYGGBACHTTYGAYTGGA-3'; designed from CPMMV sequences) for Q5294. The amplicons were directly sequenced and gave fragments of 916 bp (GenBank Accession No. MK910291) for Q5288 and 837 bp (MK910292) for Q5294. These sequences are only 71% identical. Using BLAST analyses (Zhang et al., 2000), the sequences most closely

matched CPMMV from Brazil (KC884249) at 85% identity, and CPMMV from India (AF024629) at 76%, for Q5288 and Q5294, respectively.The ICTV demarcation threshold for species within the *Carlavirus* genus is less than 72% identity between coat protein or polymerase sequences. A comparison of the partial coat protein nucleotide sequences indicated the Australian isolates fall within CPMMV with similar identities to each other (78.9%) and the type species originally reported from Ghana (Brunt & Kenten, 1973) (NC_014730), 78.1% (Q5288) and 78.9% (Q5294). Further analyses reveal the CPMMV type species is very diverse from other isolates and the ICTV criteria is not met for the polymerase gene (Table 1).

This is the first report of a carlavirus infecting plants in the *Fabaceae* in Australia. The Australian isolates are genetically distinct from each other and to international CPMMV isolates. Further refinement of the taxonomy of carlaviruses infecting hosts in this genus, including these Australian isolates is required.

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Figure 3









Figure 4

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Figure 5