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 and the forward primer Carla7190F (5′-GGNYTNGGNGTICCIACIGARCAYGT-3′; designed to detect a range of carlaviruses) for Q5288 and CPMMV7277F (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.

Acknowledgements

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Figure 1

Figure 2

Figure 3

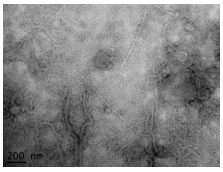

Figure 4

Table 1. Percent nucleotide identities for *Cowpea mild mottle virus* (CPMMV) isolates published on GenBank (accession numbers provided). Relationships above the diagonal are identities of whole coat protein sequences and those below are for whole RNA dependent RNA polymerase sequences. The type isolate for CPMMV is NC_014730 from Ghana. Light blue shading indicates where a relationship does not fit ICTV criteria species demarcation.

	NC_014730 Ghana 1973	MF490898 India 2015	KX682906 China 2018	KX682908 Brazil 2012	KX682907 Brazil 2012	KX682910 Brazil 2014	KX682911 Brazil 2009	KC735210 USA 2007	KX682909 Brazil 2014	KX682912 Brazil 2002	KX682914 Brazil 2009
NC_014730 Ghana 1973		73.842	78.893	78.777	78.888	78.842	78.124	79.228	79.028	78.777	78.547
MF490898 India 2015	88.132		85.858	85.856	84.428	89.455	84.349	84.246	85.480	84.128	84.775
KX682906 China 2018	88.796	73.598		89.504	89.584	88.651	89.389	89.389	85.021	89.275	89.735
KX682908 Brazil 2012	88.87	73.452	79.145		88.847	90.773	89.202	88.962	88.238	88.847	89.238
KX682907 Brazil 2012	88.865	72.247	79.844	94.724		90.541	88.902	88.735	89.455	89.519	88.731
KX682910 Brazil 2014	88.9	74.681	88.255	91.288	90.605		90.457	90.457	91.485	90.511	90.457
KX682911 Brazil 2009	89.408	74.488	88.444	90.849	87.257	97.372		89.377	89.283	88.962	89.377
KC735210 USA 2007	89.704	74.727	88.824	90.686	87.083	97.396	99.411		89.429	88.701	88.847
KX682909 Brazil 2014	89.276	74.172	88.288	86.095	87.558	97.264	98.979	98.408		86.198	88.609
KX682912 Brazil 2002	88.398	74.248	88.246	88.219	90.955	97.125	98.795	98.562	88.51		88.731
KX682914 Brazil 2009	88.611	74.422	88.316	87.220	87.609	97.123	98.885	98.586	98.584	98.728	

Figure 5

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