New Disease Reports

A distinct lineage of *Watermelon mosaic virus* naturally infects honohono orchid (*Dendrobium anosmum*) and passionfruit (*Passiflora edulis*) in Hawaii

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An orchid nursery in Honolulu, Hawaii reported honohono orchids (*Dendrobium anosmum* cv. Little Sweet Scent) with deformed flowers displaying colour break (Fig. 1) and mild chlorotic streaking of the leaves (Fig. 2). Eight orchid plants displaying these symptoms were assayed for dendrobium mosaic virus, a strain of the *Bean common mosaic virus* group of potyviruses that has been previously detected in Hawaii's honohono orchids (Hu *et al*., 1995). A potyvirus group ACP-ELISA was performed following the manufacturer's directions (Agdia Inc., Elkhart, USA), and indicated the presence of a potyvirus in these orchids. RNA was extracted from the plants using an RNeasy® Plant Mini Kit (Qiagen Inc., Valencia, USA) and universal potyvirus RT-PCR was performed (Zheng *et al*., 2010) which confirmed the presence of a potyvirus in these samples. Amplification products were cloned into pGEM®-T Easy (Promega Corp., Madison, USA) and three clones from each of five of the samples were sequenced at the University of Hawaii's Advanced Sequence Genomics Proteomics and Bioinformatics laboratory. All sequences had highest sequence similarity to *Watermelon mosaic virus* (WMV). Using the extracted RNA as template, the complete genome of WMV was amplified and sequenced using overlapping conserved primers (Table 1) and molecular cloning as described above. The sequence was deposited into GenBank (Accession No. HQ384216).

In the vicinity of the orchid nursery, passionfruit (*Passiflora edulis*) vines were observed with leaves displaying mosaic, "green islands", and severe rugosity (Fig. 3). These symptoms on passionfruit were subsequently observed at two other locations in Honolulu, and leaf samples were collected from a symptomatic plant at each location. The same ACP-ELISA and RT-PCR assays described above were performed that also indicated potyvirus infection in all three samples. Sequencing of the RT-PCR product identified the presence of WMV in these passionfruit samples. The complete genome of WMV from one of these samples was sequenced as described above and the sequence deposited into GenBank (KX512320).

The WMV present in honohono orchids and passionfruit were closely related, with a nucleotide identity of 96% over the entire genome. However, they had nucleotide identities of only 89% (WMV strain ShanXi; JX079685) to 93% (WMV strain FMF00-LL1; EU660581) to the sequences of other WMV strains present in GenBank. Phylogenetic analysis based on the full-length polyprotein sequence using a neighbour-joining algorithm placed these strains in a distinct lineage within the WMV clade (Fig. 4).

WMV (previously known as WMV-2) was reported in Hawaii in the late 1960's following a statewide cucurbit virus survey (Shanmugasundaram *et al*., 1969), however this diagnosis was based entirely on an indicator plant bioassay. Since the widespread adoption of serological assays for virus detection, there have been no reports of WMV in Hawaii, including a subsequent survey for cucurbit viruses (Ullman *et al*., 1991) as well as records from the University of Hawaii's Agricultural Diagnostic Service Center. Together, this suggests the earlier report of WMV in Hawaii may have been a misidentification. WMV is a serious pathogen of vanilla orchid (*Vanilla* spp.) in South Pacific Islands (Grisoni *et al*., 2004), but to the best of our knowledge this is the first report of WMV naturally infecting either *Dendrobium* or *Passiflora* species, and represents the first reliable report of this virus in Hawaii. Given the unusual natural host range and phylogenetic placement of the WMV strains described in this study, it is unclear whether they pose a threat to cucurbit or vanilla production in Hawaii.

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Figure 1

Figure 2

Figure 3

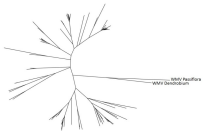

Figure 4

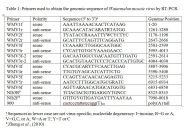

Figure 5

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