

## Confirmation of First Report of Orchid fleck virus in *Phalaenopsis* Hybrid Orchids in the USA

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*Orchidaceae* family members are diverse and are used for food, cut flowers, and potted plants. In 2013, the United States sold \$246 million of potted orchids and \$5.6 million of cut flower orchids. Sixteen *Phalaenopsis* hybrid orchids were submitted for pathogen identification in December 2014. New leaves exhibited necrotic flecks with chlorotic rings (Fig. 1 A) or necrotic lesions with leaf reddening (Fig. 1 B). Older leaves on the same plants were either asymptomatic or had large chlorotic spots. Flowers were asymptomatic (Fig. 1 C). Bacterial streaming was not observed under low magnification and very few bacterial colonies on nutrient broth yeast extract medium were isolated from symptomatic tissues. Serological immunostrip testing for *Cymbidium mosaic virus* and *Odontoglossum ringspot virus* were negative (Agdia Inc., Elkhart, IN). *Brevipalpus* sp. mites were observed by dissecting microscopy on several leaves. Transmission electron microscopy (TEM) of symptomatic leaves using partially purified extracts revealed no virus particles. TEM using a rhabdovirus extraction protocol (Doi et al. 1977) confirmed the presence of bacilliform virus particles. One characterized orchid-infecting bacilliform virus is Orchid fleck virus (OFV) (Peng et al. 2013).

Total RNA was extracted (RNeasy Plant Mini Kit, Qiagen, Valencia, CA) and tested by one-step reverse-transcription PCR (RT-PCR) with an OFV specific primer (mN2) and degenerate primer (polydT/SP6) designed to amplify an 800 base pair (bp) portion of the nucleoprotein gene within RNA 1 (Blanchfield et al. 2001). Fifteen out of sixteen plants tested positive for OFV. All positive reactions resulted in 380-bp, 800-bp, and 882-bp products (Fig. 2). These three products from two plants were gel-purified (PureLink Quick Gel Extraction Kit; Invitrogen, Waltham, MA), ligated, and cloned (pGEM-T Easy Vector System; Promega, Fitchburg, WI). Two or three clones were sequenced in both directions (UMGC, Minneapolis, MN) and the consensus sequence (Sequencher 5.1; Gene Codes Corp., Ann Arbor, MI) for the 800-bp product's 660 bp open reading frame was deposited in GenBank (Accession Nos. KR074431 and KR822590). All products were 100% identical to each other. The 660-bp ORF had 100% query cover and 99% sequence identity to reported nucleoprotein genes from OFV isolates in Australia

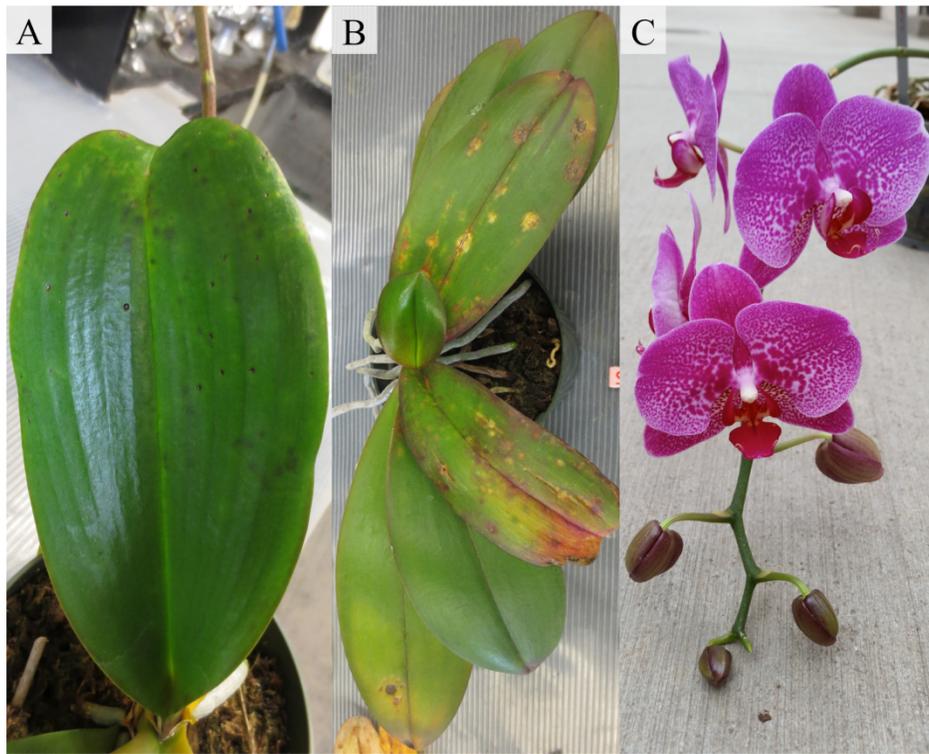
(Accession Nos. AF343871.1, AF321777.1, and AF343872.1), South Africa (AF343874.1), Germany (AF343875.1), and Brazil (AF321776.1). The 380-bp product was identical to the first 380 bases of the 800-bp product. The 882-bp product encoded the forward primer sequence, had 66% identity to *Elaeis guineensis* putative transporter ArsB (XP\_010935806.1), and was likely amplified from orchid mRNA.

Twelve asymptomatic *Phalaenopsis* hybrid orchids acquired in February 2015 from commercial growers were tested as described above for the presence of OFV. *Brevipalpus* sp. mite exoskeletons were observed by dissecting microscopy. Eleven reactions yielded a 380-bp product while one reaction had a 380-bp and 800-bp product (Fig. 3). First-strand cDNA was made using the reverse primer (polydT/SP6) and SuperScript III First-Strand Synthesis System (Invitrogen). cDNA was purified using a Pure link Quick PCR purification kit (Invitrogen) and used in a GoTaq (Promega) PCR with the mN2 and polydT/SP6 primer pair. All 12 orchids tested positive for OFV when cDNA was created first (Fig. 4). These results suggest that from these asymptomatic plants and virus isolate, the one step RT-PCR can yield false negatives.

A previous report, commonly cited as a United States OFV detection, only describes uncharacterized bacilliform virus-like particles of varying sizes observed by TEM in ultrathin sections of *Brassia* and *Cymbidium* (Ko et al. 1985). This is the first report to confirm OFV infection in the United States by microscopy and sequence analysis. OFV can be spread mechanically (Doi et al. 1977) or by the false spider mites *Brevipalpus californicus* and *B. phoenicis* in a persistent manner (Kondo et al. 2003; Hogenhout et al. 2008). It is likely that some, if not all, of the first group of *Phalaenopsis* submitted were infected with OFV at the time of purchase (from commercial growers 3 months to 5 years ago) and was then spread to any non-infected plants in the same location by the false spider mite. OFV has previously been reported in Australia, Brazil, Costa Rica, Denmark, Germany, Japan, and Korea (Blanchfield et al. 2001, Peng et al. 2013). Current management recommendations include purchasing virus-tested orchids, maintaining sterilization between plants, and testing mother plants prior to tissue culture propagation for the presence of OFV.

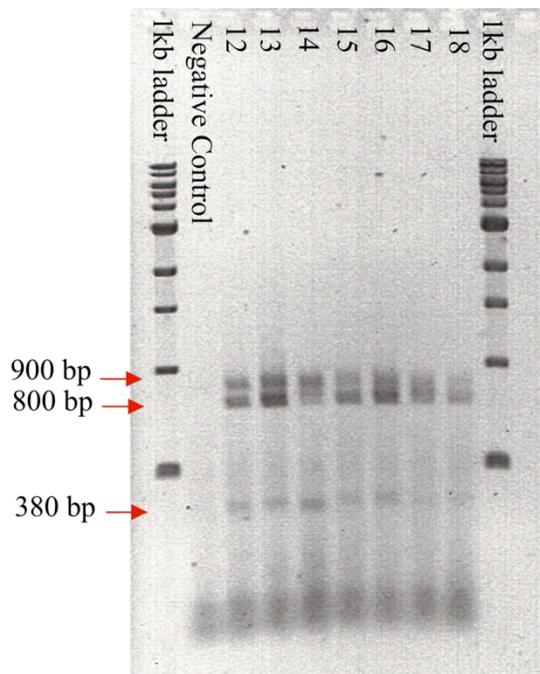
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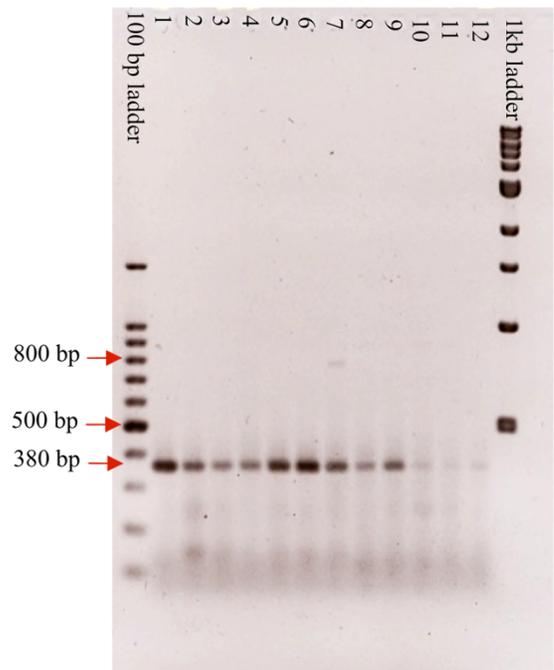
**FIGURE 1**

Symptoms of *Phalaenopsis* hybrid orchids infected with Orchid fleck virus (OFV), submitted in December 2014. The plants were tested by electron microscopy and one-step reverse-transcription PCR and were positive for OFV. **(A)** Leaves with necrotic flecks and chlorotic rings. **(B)** Leaves with necrotic lesions and leaf reddening. **(C)** Flowers were asymptomatic.



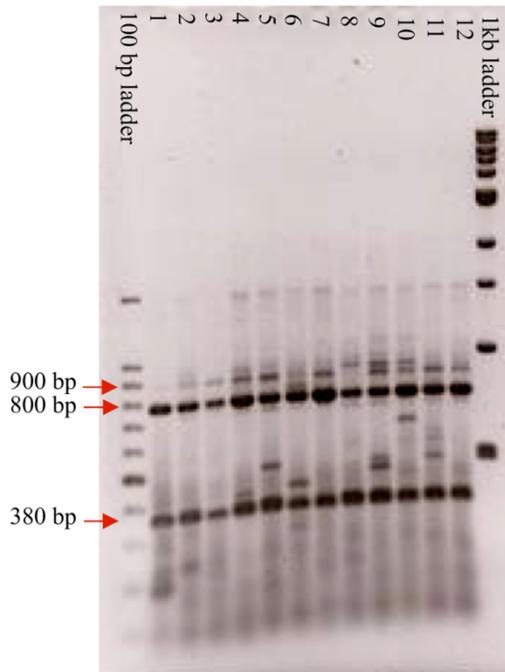
**FIGURE 2**

Symptomatic orchids numbered 12 to 18 had total RNA extracted and tested by one-step RT-PCR. The outside lanes contain a 1-kb New England Biolab ladder. The negative control reaction used sterile water. All positive reactions resulted in a 380-bp, 800-bp, and 880-bp product as the red arrows indicate.



**FIGURE 3**

Asymptomatic orchids (1 to 12) obtained from commercial producers and tested by one-step RT-PCR for the presence of OFV. The left lane contains a 100-bp Promega ladder. The right lane contains a 1-kb NEB ladder. Eleven reactions (samples 1 to 6, 8 to 12) produced a 380-bp product while one reaction produced a 380-bp and the targeted 800-bp product (sample 7).



**FIGURE 4**

Asymptomatic orchids (1 to 12) obtained from commercial producers and tested by creating cDNA and then running a PCR reaction. The left lane contains a 100-bp Promega ladder. The right lane contains a 1-kb NEB ladder. All reactions produced a 380-bp, 800-bp, and 880-bp product (along with other bands likely amplified from plant RNA). These results suggest the one-step RT-PCR reaction with these primers can yield false negatives for this isolate of OFV in asymptomatic *Phalaenopsis* hybrid orchids.

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