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First report of an emaravirus associated with witches broom disease and eriophyid mite infestations of the blue palo verde tree in Arizona

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Palo verde witches broom disease occurs primarily in the blue palo verde tree *Parkinsonia florida* (Benth. ex A. Gray) S. Wats, which is native to the southwestern (SW) states of the US and northwestern Mexico. Symptoms of witches broom disease are manifest as dense clusters of short, flexible, thornless branches with stunted leaves, and branch dieback (Figure 1). With the increased popularity of the blue palo verde as a xeriscape tree in the SW-US, the disease has spread widely in both naturally-occurring and in nursery-grown trees, at >50% incidence in nursery-grown trees, alone. Despite the first report of witches broom disease in blue palo verde trees more than 50 years ago (Werner and Olsoen, 1994) the etiology has not been determined. Here, the suspect causal agent of the disease was identified using a genomic pathology-molecular validation approach. Total RNA was purified from newly developing leaves from symptomatic (n=21) and asymptomatic (n=7) palo verde trees during 2015-2016. RNAseq libraries were constructed from total transcripts and subjected to Illumina Hi-

Seq 2500 sequencing. Sequence reads were *de novo*-assembled and annotated using BLASTn to search the GenBank sequence database. Four viral RNAs (1-4) of approximately 7, 2, 1.4, and 1.5kb were identified from symptomatic but not asymptomatic palo verde leaves. The RNAs 1-4 encoded a predicted RNA-directed RNA polymerase, envelope glycoprotein, coat protein, and movement protein respectively [RNA 1: MF766025, RNA-2: MF766030, RNA-3: MF766035, RNA-4: MF766040], and shared similarity scores ranging from 60-65% with the analogous RNAs 1-4 of Wheat mosaic virus (genus, *Emaravirus*; family *Fimoviridae*), a negative-sense, single-stranded RNA plant virus having an eriophyid mite vector (Stewart et al. 2013). Sequence-specific primers were designed and used to amplify RNAs 1-4, respectively, by reverse transcription-PCR. Sanger sequencing of the cloned amplicons (548-1465bp) confirmed the presence and absence of all four viral RNA components in symptomatic (n=4/4) and asymptomatic (n=0/4) leaf samples, respectively, collected from landscape and nursery-grown trees during 2017. Pairwise distance analysis of the partial RT-PCR amplicons and Illumina contigs determined for RNAs 1-4, shared 99.2-100% nt identity, respectively. Surveys for the presence of the eriophyid mite, *Aculus cercidi* (Keifer, 1982) indicated that it reached high population densities in symptomatic palo verde trees, however, in asymptomatic trees it was difficult to find. Although viral causality has not been demonstrated, the first discovery of an emaravirus-like genome in symptomatic blue palo verde trees heavily infested by the previously suspected eriophyid mite vector, and the absence of virus-mite complexes in asymptomatic trees, provides a robust association of an emaravirus with witches broom disease of blue palo verde, herein, proposed as Palo verde witches broom virus (PVWBV).

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References: Keifer, H.H. et al. ARS-USDA Agricultural Handbook No. 573, p.178, 1982; Stewart, L.R. Plant Dis. 97:1125, 2013; Werner, F.G. and C.E. Olson. Insects of the Southwest. p. 75-76, 1994.

70 **Figure 1.** Blue palo verde tree showing symptoms of witches broom disease, (A) thick,
71 dense branching, (B) branch die-back and necrosis, and (C) close-up of an
72 asymptomatic (left) and symptomatic (right) palo verde tree branch, respectively.

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Figure 1. Blue palo verde tree showing symptoms of witches broom disease, (A) thick, dense branching, (B) branch die-back and necrosis, and (C) close-up of an asymptomatic (left) and symptomatic (right) palo verde tree branch, respectively.

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