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Detached leaf technique procedure successful detection and evaluation of seasonal susceptibility of peach trees to *Plum pox virus* infection by the green peach aphid (*Myzus persicae* (Sulzer)).

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Abstract: A method was developed for use in a subsequent study to evaluate changes in seasonal susceptibility of orchard peach trees to *plum pox virus* (PPV) infection by aphids. To understand this, detached healthy leaves would need to be collected from peach trees in the field at different times over the growing season and evaluated for susceptibility to aphid transmission of PPV. This study examined whether virus multiplication could be detected in aphid-inoculated detached leaves and if transmission efficiency of PPV by green peach aphids to detached leaves was comparable with that for peach seedlings. Results demonstrated that transmission efficiencies of viruliferous aphids transferred to detached peach leaves subsequently maintained on an agar bed for three weeks was not significantly different from that for intact seedlings. Overlaying infected PPV plum or peach leaf segments on the healthy peach leaves with subsequent application of aphids to the infected leaf pieces provided a comparable transmission

efficiency. Reduced handling of the aphids using this method minimized the possibility of damaging the aphids and facilitated higher throughput testing. Comparable infection rates were obtained for detached leaves using either 50 or 25 viruliferous aphids per leaf. Residue of PPV was not detected by direct real time quantitative polymerase chain reaction assay (DRT-qPCR) on non-host plants probed by viruliferous aphids. The effect of short term storage temperatures pre- or post-inoculation did not significantly alter the susceptibility of peaches to PPV infection and the transmission rate. Application of the leaf overlay method to evaluating seasonal changes in susceptibility is the subject of an ongoing study.