Examination of the evidence that seeds of *Poncirus* genus and hybrids may transmit *Candidatus* Liberibacter species, the causal agent of citrus greening

Supplement to the global pest list of Citrus spp. pathogens and an examination of evidence for seed transmission

The genus *Poncirus* is considered one of the 'true citrus fruit trees', and was originally included in the *Citrus* genus (Krueger and Navarro, 2007). In the early 1900s *Poncirus* was separated from the *Citrus* genus due to several morphological and phonological differences (Krueger and Navarro, 2007). The *Poncirus* genus currently has two known species, *P. trifoliata* and *P. polyandra* (Agusti et al., 2014). *Poncirus* shares many common traits with *Citrus* including characteristics of the fruit and seeds (Jackson, 1999). *Poncirus* is able to successfully hybridize with *Citrus* and is often used as root stock to produce several commercially important hybrids (*e.g.* citranges- *P. trifoliata* x *C. sinensis*; citrumelos- *P. trifoliate* x *C. paradise*; citrandarins- *P. trifoliata* x *C. reticulate*; citremons- *P. trifoliata* x *C. limon*; citradias- *P. trifoliata* x *C. aurantium*) (Krueger and Navarro, 2007; Jackson, 1999). *Poncirus* spp. are deciduous and considered cold-hardy enough that they can survive freezes (Saunt, 2000). Hybrids are typically less cold tolerant than pure *Poncirus* spp., but more cold tolerant than pure *Citrus* species (Saunt, 2000). In addition to increased cold tolerance, *Poncirus* and its hybrids are more resistant, or tolerant, to many plant pathogens (Saunt, 2000; Timmer et al., 2000). For example, citrumelos are reported to be tolerant of citrus nematodes, some *Phytophthora* species, *Citrus tristeza virus* (CTV), cachexia and excortis, and apparently have low rates of blight incidence (Castle and Gmitter, 1999). However, it is noted that resistance is not always absolute. For instance, there are reported isolates of CTV that can overcome the resistance expressed by *P. trifoliate* (Hilf, 2005; Harper et al., 2010).

Early observations of citrus greening (CG) (causal agents-'*Candidatus* Liberibacter asiaticus', '*Ca.* Liberibacter americanus', and '*Ca.* Liberibacter africanus') in *Poncirus* plants vary in disease severity and may lack distinct CG disease symptoms (e.g. McClean and Schwarz, 1970; Miyakawa, 1980; Nariani, 1981). More recent studies have confirmed that a number of *Poncirus* hybrids appear to display some tolerance to CG (Albrecht and Bowman, 2011; Boava et al., 2014; Boscariol-Camargo et al., 2010).

In addition to noted tolerance to *Ca.* Liberibacter spp., *P. trifoliata* may also demonstrate some resistance to Asian citrus psyllid (ACP) colonization. Hall et al. (2015), demonstrated through a series of experiments that a number of accessions of *P. trifoliata* exhibited resistance to ACP colonization. The factors that influence *P. trifoliata* resistance to ACP oviposition are unknown. Based on current data, *Poncirus* and its hybrids may display some tolerance and resistance to *Ca.* Liberibacter spp. and ACP, but it is not immune to either species. While these properties may lower the incidence or severity of CG infections, they may still occur.

Little research on seed transmission is available for most *Ca.* Liberibacter species. Most of the seed transmission research has focused on *Citrus* hosts, with the exception of three studies that used *Poncirus* or *Poncirus* hybrids along with *Citrus* spp. (Benyon et al., 2008; Benyon et al., 2009; Van Vuuren et al., 2011). In these studies, there was no significant difference in responses between *Poncirus* and *Citrus* hosts tested. *Poncirus* and *Citrus* fruit and seeds share similar characteristics (Jackson, 1999), therefore, the dynamics necessary for seed transmission (or lack thereof) are likely the same between the genera.

In unpublished research examining *Ca.* Liberibacter asiaticus cells in the vascular bundle of *Poncirus* x *Citrus* hybrid seed coats, the incidence of seeds with *Ca*. Liberibacter asiaticus colonized vascular bundles varied, but when detected the seeds vascular bundles always reflected very low populations of *Ca*. Liberibacter asiaticus (Hilf, 2015). While it is unknown what the minimum number of *Ca.* Liberibacter asiaticus cells are needed to start a new infection, it is unlikely to occur at this low levels of cells as seed transmission is not reported to occur with much higher population levels (Hilf, 2011). The current research on seed transmission of *Ca.* Liberibacter species indicates that any cell transmission from seeds to the seedling is transient, and while abnormal growth pattern may be observed in seedlings, there are no instances of seedlings developing citrus greening, (e.g. Hartung et al., 2010; Albrecht and Bowman, 2009; Shokrollah et al., 2009; Hilf, 2011; Graham et al., 2011; Van Vuuren et al., 2011). While much is still unknown about the biology of *Ca*. Liberibacter spp., there are indications that the bacteria associated with the seed coat may not all be viable. Hilf (2015) reports that the viability of *Ca*. Liberibacter asiaticus cells found in these vascular bundles is low, especially in seeds from fruit collected later in the season.

Based on the above evidence, *Poncirus* spp. and its hybrids may be less likely to be naturally infected with *Ca.* Liberibacter spp. and if plants are infected, it is unlikely that any *Ca.* Liberibacter spp. are able to cause disease through seed transmission.

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