



Reliable Sampling Tissue and Seasonality for Consistent Detection of 'Candidatus Liberibacter Asiaticus' by qPCR

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Body of Text

Huanglongbing (HLB) is the most serious disease of citrus, putatively caused by the bacterium '*Candidatus Liberibacter asiaticus*' (CLAs), transmitted by the Asian citrus psyllid (*Diaphorina citri* Kuwayama) in the USA. Most commercial citrus cultivars are susceptible to the disease, particularly conventional sweet oranges and grapefruit, decline severely in health and productivity (Bové 2006, Gottwald 2010). Early detection and prompt response are key factors in the eradication or suppression of HLB epidemics in California. Real-time PCR is the standard regulatory method for CLAs detection and it can detect as little as a single copy of bacterium in a sample. However, due to lack of visible symptoms, low titer and uneven distribution of CLAs in a tree, selecting the best sample is a major hurdle for early detection by qPCR. Regulatory testing of CLAs by qPCR stipulates sampling of mature leaves. Such leaves are good for visual inspection of HLB symptoms but can be unreliable tissue for early detection and low titer infections. Symptoms of HLB develop slowly and without symptoms, collecting that right leaf/shoot amongst >200,000 estimated leaves per mature tree is practically impossible for early diagnosis.

The pressure flow hypothesis or Münch mechanism states that sugars, made in the leaves via photosynthesis, move passively down a concentration gradient, or actively transported in some cases (Fig 1). Accumulation

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of sugar (photoassimilates) in phloem osmotically attracts water, increasing the pressure in the sieve tubes at the source. This pressure pushes phloem sap towards a region of lower pressure, consequently, the sap moves by bulk flow, down a pressure gradient (Jensen *et al.*, 2016). Phloem-limited plant pathogens move in a source-to-sink fashion along with photoassimilates (Bendix and Lewis, 2018). New flush has been the reliable tissue to test *Citrus tristeza virus* (CTV) and Citrus Pest Detection Program (CPDP) of Central California Tristeza Eradication Agency has been utilizing the flush tissue for large-scale field survey for the last three decades or so (Gottwald and Hughes, 2000). Recent findings indicate new flush can be used for reliable detection of CLAs (McCollum, unpublished).

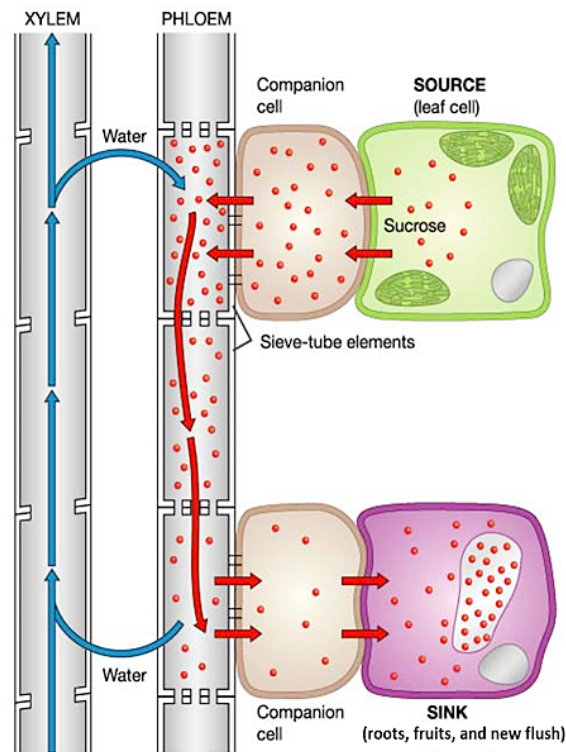


Fig. 1: Translocation of sugars (photoassimilates) in plants. Pressure flow hypothesis/ Münch mechanism by which sugars are transported through the phloem, from sources to sinks tissues

Bar-Joseph *et al.*, (1979) found higher content of CTV in peduncle bark than the bark of branches of same age. Similarly, our preliminary data of testing *Spiroplasma citri* from young shoot indicates that the titer of this phloem-limited bacteria is more in peduncle of young fruit than the bark of young shoot and petiole of young leaf (Hajeri and Yokomi, unpublished). Moreover, fruit tissues such as columella and receptacle are routinely used for reliable detection of *S. citri* by CPDP (Yokomi *et al.*, 2008). In Florida, Tatineni *et al.*, (2008) and in Texas, Kunta *et al.*, (2014) observed a relatively high titer of CLAs in fruit peduncle. Recently, in Texas, Park *et al.*, (2018) have shown that the CLAs can be detected in roots before appearance of visible symptoms aboveground. Moreover, CLAs titer was not only greater in the roots but also uniformly distributed, and more consistent through the year when compared to leaf samples (Johnson *et al.*, 2014, Louzada *et al.*, 2016, and Park *et al.*, 2018). In all the cases, the sink tissue such as the new flush, fruit and roots showed high titer of phloem-limited citrus pathogens. In Florida, Irey *et al.*, (2011) observed that the greatest percentage of HLB positives by qPCR were during July through January, coinciding with the period of maximum leaf symptomatology and concluded that seasonality of sampling is critical.

The outbreak of HLB in California is delimited to residential properties in four southern California counties: Los Angeles, Orange, Riverside and San Bernardino; whereas, no detection of HLB has occurred in any commercial citrus orchard in California. Rapid detection and eradication of infected trees is the highest priority along with monitoring and control of *D. citri*, vector of CLAs in commercial orchards of California.

Conclusion

Early detection of CLAs, long before symptom appearance, is a critical need in California. Moreover, regulatory detection currently requires direct pathogen detection and quantitation of target DNA of CLAs. When it comes to sampling large trees in commercial setting, data gap exists despite valuable data available from previous studies. There is a need for systematic sampling study to identify the most reliable tissue matched with its ideal season for early detection of CLAs in mature citrus trees.

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