

## **Lack of Evidence for Seed Transmission of '*Candidatus Liberibacter africanus*' Associated with Greening (Huanglongbing) in Citrus in South Africa**

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'*Candidatus Liberibacter africanus*' is associated with citrus greening (huanglongbing [HLB]) in South Africa. Various unpublished reports have suggested that the related bacterium '*Ca. L. asiaticus*' associated with HLB in citrus might be seed transmissible based on real-time PCR results. Seed transmission poses a risk of long distance disease spread, especially with the dissemination of rootstock seed. Therefore, it was essential to determine whether '*Ca. L. africanus*' is seed transmitted in citrus. Fruit from 26 '*Ca. L. africanus*'-infected branches of six citrus cultivars showing greening symptoms were collected and the seed was harvested. Cultivars included were Minneola tangelo (*Citrus reticulata* × *C. paradisi*); sweet oranges (*C. sinensis*) Premier midseason, Clanor midseason, and Olinda Valencia; Eureka lemon (*C. limon*) and Troyer citrange (*Poncirus trifoliata* × *C. sinensis*) rootstock variety. Branches bearing each fruit were collected and confirmed to contain '*Ca. L. africanus*' by real-time PCR testing using Taqman probe HLBp and HLBaf and HLBbr primers as described by Li et al. (3). The seed of each sample was sorted into five categories ranging from healthy looking to totally aborted based on their appearance before planting. Germination was done in seed trays under vector-free conditions at 24 to 28°C. Thereafter the seedlings were planted in small, plastic bags and monitored for greening-like symptoms or other abnormalities for up to 2 years. A slow-release, balanced fertilizer was applied and supplemented with micro-nutrient sprays for plant maintenance. Plants showing abnormal symptoms were potted into larger pots and closely monitored. These samples and a number of other seedlings showing growth abnormalities were tested for '*Ca. L. africanus*' by real-time PCR as described above. In total, 1,570 seedlings were obtained. Some abnormal symptoms such as small chlorotic leaves, interveinal chlorosis, yellow veins, and stunting were seen in some seedlings. Most symptoms resembled deficiencies, and no blotchy mottle typical of '*Ca. L. africanus*' infection was noted on any of the seedlings. Abnormal seedlings arose from normal and abortive seed. One hundred and eighteen of these seedlings (8 Minneola tangelo; 24 Premier midseason, 42 Clanor midseason, 33 Olinda Valencia, and 11 Troyer citrange seedlings) were individually tested using real-time PCR for

'*Ca. L. africanus*' detection. These seedlings had germinated from essentially healthy-looking seed (category 1) to seeds with severe abnormalities (category 5) and 33, 24, 23, 30, and 8 seedlings, respectively, were tested from each of the five seed categories. No samples tested positive with real-time PCR based on a positive/negative threshold Cq value of 35. Buds of some seedlings that yielded the lowest Cq values above 35 were grafted onto healthy 'Madam vinous' sweet orange (*C. sinensis*) seedlings and monitored for symptom development for 3 months. No symptoms developed and all these indicators also tested negative for '*Ca. L. africanus*', indicating the absence of a transmissible agent. Just as other researchers (1,2) have recently indicated a lack of evidence for seed transmission of '*Ca. L. asiaticus*', no seed transmission of '*Ca. L. africanus*' could be demonstrated in this experiment either.

*References:* (1) U. Albrecht and K. D. Bowman. HortScience 44:1967, 2009. (2) J. S. Hartung et al. Plant Dis. 94:1200, 2010. (3) W. Li et al. J. Microbiol. Methods 66:104, 2006.