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Heat treatment of Huanglongbing –affected citrus trees in field for reduction of “*Candidatus Liberibacter asiaticus*”

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Huanglongbing (HLB, yellow shoot disease) is a devastating citrus disease worldwide. Research conducted by Lin Kung-Hisang et al. in 1960s China suggested that heat treatments were effective at eliminating HLB pathogen in scions. We tested the effect of high temperatures on the reduction of “*Candidatus Liberibacter asiaticus*” (Las) titers in HLB-affected citrus trees in Guangdong, China. Heat treatments were delivered via covering a tree with a temporary enclosed tent of plastic sheeting, which used natural sunlight to raise ambient temperature. Twenty-four sweet orange trees with HLB symptoms in an orchard were selected and divided into six blocks with three blocks of four trees, each receiving heat treatments, and the other three blocks used as controls. Heat treatments were performed three times in August with temperature exceeding 38°C for three hours each time. Leaf samples were collected in the following months and real-time PCRs with primer set HLB_{asf}/HLB_{asr} and TaqMan probe HLB_p were used to monitor Las titer changes.

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Gas exchanged and water relations of ‘Valencia’ oranges trees infected with Huanglongbing (HLB, ex greening)

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Symptoms of plants infected with HLB (causal agent “*Candidatus Liberibacter* “spp.) as part of the leaves yellowing and mottling, indicate that their photosynthetic capacity may have changed. Obstructions in the phloem, common in the infectious process, may also affect photosynthesis and water relations. In this study we verified whether HLB affects gas exchange and water relations in two phases of development of symptoms in ‘Valencia’ oranges trees, grafted in Rangpur, with 12 years of age. The treatments consisted of (a) leaves of symptomatic patients, (b) leaves of asymptomatic patients (both PCR) and (c) leaves of plants PCR -. Evaluations were performed in December 2011 (onset of symptoms) and March 2012 (overt symptoms). We investigated the rate of CO₂ assimilation (*P_n*), transpiration (*E*), stomatal conductance (*g_s*), water use efficiency (*WUE*) and the instantaneous efficiency in the use of CO₂ (*P_n/C_i*) three times (8:00, 12:00 and 15:00) using a Li-IRGA 6400. The water potential (ψ), measured on a PMS pressure chamber, was observed at 6:00 and 14:00 hours. The results showed that *P_n* and the *WUE* were not affected, but there was a reduction of *E*, during the hottest hours of the day, probably related to lower *g_s* and ψ . The *P_n/C_i* was somewhat reduced in infected plants, only in March 2012. The maintenance of photosynthetic capacity and changes in water balance are possibly related to the obstruction of the phloem and to the damage of the plant root system.

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Assessing plant health risk in relation to *Xanthomonas citri* strains causing citrus bacterial canker and evaluating measures for managing this risk

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In the frame of a project funded by the European Food Safety Authority (Prima Phacie), effort was put into identifying and testing qualitative plant-pest risk assessment schemes for their suitability in supporting risk management decisions for the European Union. Five schemes were tested, two largely based on the EPPO

scheme and three adapted from schemes used in non-European countries. We report the results from the application of these schemes as applied to *Xanthomonas citri* strains causing Citrus Bacterial Canker, in regard to the risk of its entry, establishment and spread, as well as its potential impact. For this pathogen, three entry pathways into the EU risk assessment area were considered: a) import of fresh citrus fruits, b) import of ornamental rutaceous plants or plant parts, and c) illegal entry of plant propagative material. With the current EU measures in place, of the five schemes tested, two indicated path (c) as that of the highest risk, whereas the other three suggested path (a) as such. This discrepancy is due to the different level of details the components of the risk of entry are considered in each scheme. Most schemes suggested that the establishment potential lay around the mid-range of possible values. All schemes indicated a high rate for potential spread (primarily through human activities) and a medium to high rate for impact potential. The effectiveness of risk management measures was evaluated by comparing results of assessments with and without management measures in place.

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Characterization of a new *Xanthomonas citri* subsp. *citri* isolate which triggers a host-specific response

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Citrus is an economically important fruit crop that is severely afflicted by Asiatic Citrus Bacterial Canker (CBC), a disease caused by *Xanthomonas citri* subsp. *citri* (XC). Recently, we have identified a XC strain, named XCT44, which induces a hypersensitive-like response in *Citrus limon* leaves. To gain insight into the dynamics of these host-specific responses, we have investigated biofilm formation and accumulation of defense markers by XCT44 on different citrus species. Biofilm formation was monitored by confocal laser scanning microscopy (CLSM) over a 10-day period. XCT44 strain was unable to form structured biofilm after 6 days post-inoculation. This observation is associated with early production of reactive oxygen species by the plant. Transcriptomic analysis of *C. limon* leaves in response to the non pathogenic (XCT44) and pathogenic (XC) strains showed that XCT44 induces specific genes that points towards the involvement of salicylic acid and glucosinolates metabolism in the onset of the defense responses. Further characterization of these genes with a role in this HR-like response will be important for the development of new strategies to manage the disease.

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Analysis of genetic diversity of *Xanthomonas citri* subsp *citri* strains. Characterization of a new isolate which triggers a host-specific response

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Citrus is an economically important fruit crop that is severely afflicted by Asiatic Citrus Bacterial Canker (CBC), a disease caused by *Xanthomonas citri* subsp. *citri* (XC). Argentina is one of the world's largest citrus producers, accounting for 17% of global lemon supply, with the majority of production concentrated in the northwestern province of Tucuman. To gain insight into the genetic diversity of *Xanthomonas* causing CBC in Tucuman, a total of 42 *Xanthomonas* isolates were collected from different citrus species across seventeen different orchards and subjected to molecular, biochemical and pathogenical tests. Analysis of genome-specific XC markers and DNA polymorphism based on rep-PCRs showed that all isolates belonged to XC. Thirty-eight haplotypes with relatively high genomic inter-relationship compared to XC reference strains were discriminated. Pathogenicity tests showed that one isolate, named XCT44, which shares 91% genetic similarity to the reference strain XCT, has specific host range. XCT44, induces a hypersensitive-like response in *Citrus limon* and *Citrus paradisi* leaves and weak canker lesions in *Citrus aurantifolia* and *Citrus clementina*