



Struggling Citriculture in Florida is a Source for Innovation in Horticultural Science

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Short Commentary

Citrus is one of the most grown fruit crops in the planet, and has very important economic value for several regions across the tropics and subtropics. These include countries in the Northern Hemisphere such as United States of America and Mexico in the Americas, Spain, Italy, Egypt, Turkey and Israel in the Mediterranean, China, India and Japan in Asia, and countries in the Southern Hemisphere such as South Africa, Australia, Brazil, Uruguay and Argentina. This distribution of Citrus culture between both hemispheres warrants transport of fruit between regions on a seasonal basis through regulated trade. However, with commercial transport of commodities and increasing traffic of passengers among countries, new threats have appeared such as the spread of diseases. A dramatic example of this is the spreading of the disease known as huanglongbing (HLB), the yellow dragon disease. HLB is the most economically devastating disease of citrus worldwide, it was described for the first time in southern China in 1919 and is now known to occur in about 40 different countries around the globe. The HLB bacterium belongs to the genus *Candidatus Liberibacter*. Currently three species are known, *Candidatus Liberibacter asiaticus*, occurring in Asian countries, Brazil and the USA, *Candidatus Liberibacter africanus* with its subspecies "capensis", recorded from African countries, and *Candidatus Liberibacter americanus* present also in Brazil [1]. It has been suggested that each liberibacter species has evolved in the continent after which it is named. HLB symptoms are virtually the same wherever the disease occurs. HLB can be transmitted by grafting from citrus to citrus and by dodder to periwinkle [2]. The psyllids *Trioza erytreae* and *Diaphorina citri* are natural vectors [1].

In Florida, the disease is caused by the fastidious gram-negative α -proteobacterium, *Candidatus Liberibacter asiaticus* (CLAs), and since it was first detected in 2005 it has reduced the citrus acreage and fruit production by 70% [3]. CLAs propagates within the phloem of citrus plants producing root loss, branch die-back, yellow shoots, blotchy mottle chlorotic patterns on leaves, and off-tasting and malformed fruit. HLB infection inhibits root growth, reduces nutrient uptake, and promotes leaf and fruit drop, and whole tree decline that is often lethal. HLB symptoms include reduced growth, less concentrated bloom, leaf chlorosis, and loss of root mass [4,5]. Regarding citrus fruit production, two main effects are associated with HLB: increased preharvest fruit drop, and loss of internal quality. Both effects reduce substantially economical returns for growers. The mechanism by which CLAs causes HLB is still not well determined [6-9]. Currently there is no cure, and disease management consists of controlling psyllid vector with regular insecticide applications, removal of infected trees, elimination of affected groves, and enhanced nutrition programs for improving plant health of infected trees. However, results are not

consistent in neither enhancing tree health and fruit yield and quality nor stopping or slowing infection rate, and now it is estimated that more of 80% of Florida citrus acreage is infected [3].

The impact of HLB in different parts of the world has devastated the Citrus Industry. With no cure on the horizon, the solutions currently available are focused on prolonging tree lifespan and trying to maintain fruit yield. However, this threat has sparked a massive research effort that is producing a significant amount of innovation. In this Commentary, I will focus in some of the most promising approaches currently developed and adopted in Florida.

Breeding, gene engineering and gene editing

Citrus breeding of superior varieties is not an easy task and almost impossible to achieve by conventional sexual hybridization, and requires other techniques such as somatic hybridization [10]. However, searching and breeding for new varieties with tolerance/resistance to HLB and other stresses and diseases are critical to maintain the Industry alive. With the sequencing of the citrus genome and elucidation of its fascinating evolutive history [11], now we have unique tools to breed new varieties and look for desired traits in a targeted manner, like for example introgressing HLB tolerance/resistance genes from trifoliolate oranges into edible, commercial varieties [12]. Since traditional breeding of citrus varieties is challenging due to different constraints that include polyploidy, polyembryony, extended juvenility and long crossing cycles, targeted genome editing technology has the potential to shorten varietal development for some traits, including disease resistance [13]. A great example of precise genome editing is the technique known as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and its associated Cas9 protein. Through this approach, it has been possible to confer resistance to canker infection in citrus by modifying an effector binding element in the promoter region of one single allele of the canker susceptibility gene CsLOB1 [14] or in both alleles of the gene in Duncan grapefruit [13]. This technique shows great promise to attack HLB as well. Certainly, long lasting solution will be the development of citrus cultivars resistant to disease through engineering, and other strategies based on overexpression of pathogenesis related protein PR1 have also shown some promise [15].

New planting/managing methods

There is still a lot that can be achieved in the area of grove management. Under HLB pressure, different management strategies are being assayed. One of the most dramatic effects of HLB infection is the loss of root density. Nutritional stress in citrus trees due to low availability of nutrients and roots loss directly influences fruit yield and

crop value by aggravating fruit drop and reducing overall quality. Improving root health by lowering high soil pH to make nutrients available to the root system improves performance of HLB affected trees [16]. In addition, preliminary studies indicate that various fertilizer formulations may improve health of infected trees [17] and now different formulations are being assayed in Florida. Another example of improved management under HLB deals with grove architecture. Higher density plantings will be fundamental to achieve higher economic yields through maximize fruit yield per acre, minimize the use of all production inputs per acre and reduce the environmental impacts of growing citrus [18]. Key factors for the new high density model of grove will be selecting of dwarfing rootstocks, managing tree row structure as a hedge and adopting mechanized harvesting [19]. Probably one of the most breakthrough examples of the change in paradigm that HLB has brought to citrus grove management comes from the adoption of CUPS (Citrus Under Protective Screens) systems. CUPS completely exclude the asian citrus psyllid, *Diaphorina citri* and therefore the disease, and is specially indicated from growing citrus for the fresh market, due to higher economic returns of this kind of fruit, since the costs to establish the greenhouse structures is very important. However, the benefits of eliminating HLB are immediate and include normal tree growth, higher yields of quality fruit, negligible fruit drop, and uncomplicated fertilizer and irrigation requirements [20].

New treatments

Research efforts under HLB immense pressure are resulting in novel treatments consisting of creative applications of known technologies, chemicals such as bactericides or plant hormones such as brassinosteroids that show great promise for controlling not only HLB but other diseases. Since the list of these promising compounds and techniques is expanding at a fast pace under the urgency created by HLB, I want to highlight here only two examples of them that have sparked great interest in Florida: The first technology consists of nano-oxide materials (known as Zinkicide). These nanoparticles are able to control certain diseases such as canker in citrus better than conventional copper-based methods and present advantages such as lower toxicity, higher antimicrobial activity and ability to move translaminal in the plant [21]. Recently, it has been shown by cuban researchers the potential of a relatively newly described group of hormones, brassinosteroids, for controlling HLB both in already infected plants and with potential as a protective treatment measure for nursery plants before planting in the grove. Brassinosteroids were able to reduce significantly bacterial titer and alleviate symptoms of greening in HLB-affected mature citrus trees [22]. This effect seems to be mediated by the activation of a high number of defense-related genes, including jasmonic acid signaling pathway and downstream salicylic acid-related signaling genes such as pathogenesis-related PR1 and PR2. However, the long-term effects of this treatment on tree health and fruit yield and quality have not been shown yet and research is currently undergoing.

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