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# Field identification of Huanglongbing (HLB) and its management alternatives

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## ABSTRACT

**Objective:** The objective of this investigation is to identify huanglongbing (HLB) in the field and know their control alternatives.

**Design/methodology/approach:** Was investigated the introduction of the phytopathogen *Candidatus Liberibacter*, symptoms and control of HLB. The generally observed symptoms of this disease are irregular yellowing in the leaves and reversed ripening on the fruit.

**Results:** According to various authors, an integrated control of the disease must be carried out, such as pest control, production of seedlings in certified nurseries, a suitable nutrition management and working together with educational and research institutions.

**Limitations on study/implications:** HLB or citrus greening is the most devastating disease of citrus trees which has caused millions of dollars in losses worldwide. However, although there are several research, there is still no established cure.

**Findings/conclusions:** Research such as that carried out by geneticist Hailing Jin at the University of California Riverside can give hope for the cure or control of this disease, however, there are limitations to this product since it is not yet commercialized.

**Keywords:** Identification, Huanglongbing, control, citrus crops.

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## INTRODUCTION

Citrus farming is an agricultural and economic activity of great global importance, (Liu *et al.*, 2012), since citrus fruits are produced in more than 140 countries, mainly in China, Brazil, India, and Mexico (FAOSTAT, 2021). However, one of the limitations to production is the damage caused by diseases, particularly Huanglongbing (HLB), commonly known as citrus greening (Sáenz-Pérez *et al.*, 2019). HLB is the most devastating disease of citrus trees worldwide since it reduces the production and quality of the fruit, resulting in economic losses (Bove, 2006), which vary according to the crop. For example, losses of up to 42, 62, and 17.3% have been reported in oranges, Mexican lime, and Persian lime, respectively (Mora-Aguilera *et al.*, 2014). In 2013, HLB was present in 39 countries; however, by 2019, it was reported in 71 countries, including the main citrus producers (CABI, 2019; EPPO, 2019). There are different types of *Candidatus Liberibacter*, depending on the country: *Candidatus*

*Liberibacter asiaticus* is present in Asia, Africa, Oceania, and the Americas, (CABI, 2019); *Candidatus Liberibacter africanus* can be found in Asia and Africa; *Candidatus Liberibacter americanus* has been reported in the Americas (EPPO, 2019); and *Candidatus Liberibacter caribbeanus* is found in Colombia (Keremane *et al.*, 2015).

## MATERIALS AND METHODS

First, literature about HLB disease was reviewed, placing special emphasis on its symptoms and management alternatives. Subsequently, a visual monitoring of citrus orchards was carried out in Tamaulipas, identifying symptoms, and taking photographs in the field.

## RESULTS AND DISCUSSION

### How the HLB infects the tree

HLB is caused by the *Candidatus Liberibacter* spp. bacteria and is mainly transmitted by the *Diaphorina citri* vector (Zhang *et al.*, 2014). *Candidatus Liberibacter* spp. can be found in the hemolymph and salivary glands of the insect vector and it enters the citrus tree through the stylet of the psyllid when the vector sucks the sap. It then moves through the sieve tubes of the phloem, until it reaches the root, where it replicates and subsequently spreads to the rest of the tree (Johnson *et al.*, 2013).

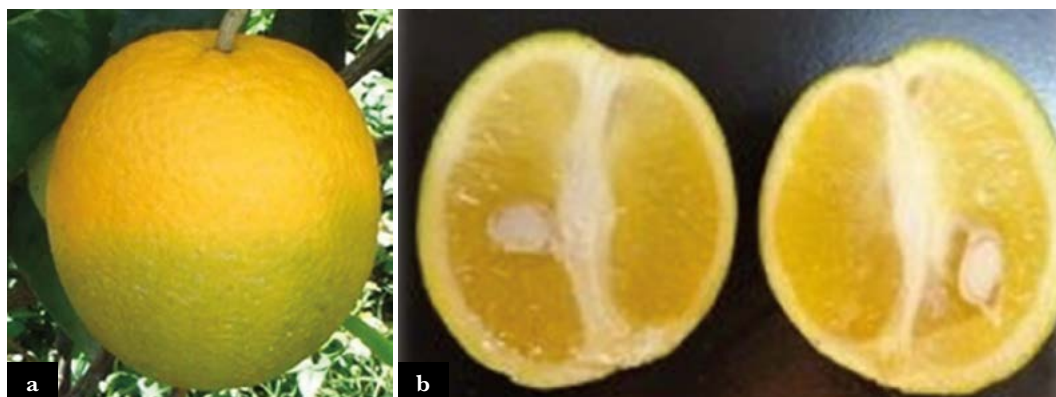
### HLB symptoms

The characteristic symptoms of HLB in citrus are the yellow, asymmetrical mottling of the leaves and growth retardation in infected plants. HLB can be identified through sporadic spots in the leaves and the lack of a similar pattern when both leaf blades are folded (Figure 1). Nevertheless, it can be confused with nutritional deficiencies; therefore, a proper identification is required (Zhi *et al.*, 2021). The leaves may also show a corky appearance and dilated veins (Bove, 2006).

Fruits infected with HLB may be smaller and asymmetrical and show inverse ripening (Figure 2), thickening of the pericarp (peel), and seed abortion, which decreases fruit quality and affects its flavor. The reduction of its organoleptic quality nullifies fruit trade in the industry (Bove, 2006). This disease also causes premature leaf drop, fruit abortion, and, in advanced stages, the death of the citrus tree (Wang *et al.*, 2017).



**Figure 1.** HLB symptoms in leaves. The irregular yellowing changes as the disease progresses.



**Figure 2.** HLB symptoms in fruit: a) inverse ripening and b) asymmetry.

### HLB management alternatives

There are various alternatives for the management and prevention of HLB, but there is still no cure for trees infected with this disease (Gottwald *et al.*, 2007).

#### Vector management

Although *Diaphorina citri* is the main vector of the *Candidatus Liberibacter* bacterium (Hall, 2018), it can also be spread by *Trioza erytreae* and other vectors (Zhang *et al.*, 2014). Since this bacterium is found in the salivary glands of the insect, it enters the tree through the stylet of the psyllid when the vector sucks the sap (Johnson *et al.*, 2013). Therefore, managing these vectors is the main strategy for HLB management (Ruiz-Galván *et al.*, 2015).

The following types of vector management are available:

1. Cultural management: providing adequate nutrition to make the plant resistant to pathogens and pests (Velasco, 1999), detecting insects before they cause damage, and promptly identifying infected plants (Mann *et al.*, 2018).
2. Biological management: using natural predators of *Diaphorina citri*, including the *Cycloneda sanguinea*, *Chilocorus cacti*, *Exochomus cubensis*, *Scymnus distinctus*, *Chrysopa* sp., and *Ocyptamus* sp. ladybugs (Miranda *et al.*, 2008), or parasitoids wasps, both ectoparasitoids (*e.g.*, *Tamarixia radiata*) and endoparasitoids (*e.g.*, *Diaphorencyrtus aligarhensis*) (Grafton-Cardwell *et al.*, 2013). Entomopathogenic fungi capable of infecting the insect vector and subsequently causing its death can also be used, including *Isaria fumosorosea* and *Beauveria bassiana* (Saldarriaga *et al.*, 2017).
3. Chemical management: the most popular control method among producers (Tiwari *et al.*, 2011). Nevertheless, these products should only be applied before the maximum population peaks, which are usually recorded at the beginning of the year, when favorable conditions for the population increase of the vector are reported (Cortez *et al.*, 2010). The most common insecticides are temik, imidacloprid, dimethoate, chlorpyrifos, cypermethrin, malathion, deltamethrin,  $\beta$ -cyfluthrin, spirotetramat, spinetoram, oxamyl, tricarboxylic acids, omethoate, sulfoxaflor, and mineral oils, among others (Orozco and Cano, 2012).

### **Use of antibiotics**

In Florida, United States, streptomycin sulfate, oxytetracycline hydrochloride, and oxytetracycline calcium complex have been approved as treatments against HLB. These foliar antibiotics are applied as aerosols. Nevertheless, no studies have proven the benefit of these compounds (Wang *et al.*, 2017).

### **Thermotherapy management**

Thermotherapy seems to be an effective method for HLB management, at least in greenhouse and growth chambers. Fan *et al.* (2016) exposed mandarin orange seedlings infected with HLB to a temperature of 45-48 °C for 4 h, 1 day a week, for 3 weeks in a row. As a result, the phytopathogen decreased by 30 to 55% and the control increased by 300%. However, the effectiveness of thermotherapy depends on both the components of the plant and the soil. This is an expensive and slow alternative in the field, although equipment is being developed to make it feasible on a larger scale (Trotochaud and Ehsani, 2016).

### **Alternative management**

Scientists at the University of California, Riverside, found a peptide that can control citrus greening. Geneticist Hailing Jin determined that this peptide is stable outdoors, withstands high temperatures, is safe for humans, and reduces the pathogen to a considerable degree; however, it is not yet commercialized, and more research is required before it can be used (Bernstein, 2020).

### **Management with the addition of nutrients**

In agriculture, fertilization covers the nutritional requirements of the plants, providing quality to the production. Likewise, it can also be used to manage diseases (Huber, 1989; Fageria *et al.*, 1997); however, a nutritional balance must be first carried out, to establish the resistance of the plant to different pathogens (Velasco, 1999), since nutritional excess or deficiencies have been proven to be able to modify susceptibility to diseases (Velasco, 1999; Huber, 1978). According to Giles (2011), macronutrient and micronutrient foliar sprays increased the vigor and productivity of citrus trees infected with HLB. Despite their high quantities, the nutrients in the soil do not meet the requirements of the crop, since some elements cannot be assimilated by the plant; this deficiency can only be compensated by the application of fertilizers (Zúñiga, 2013). Plants require at least 17 essential elements for their development, growth, and metabolism, including O, H, and C, which they obtain from H<sub>2</sub>O, CO<sub>2</sub>, and air. The rest are mineral nutrients, which are classified as micronutrients or macronutrients, according to the amount absorbed. Macronutrients are found in higher concentration in plant tissue; they are divided into primary elements, such as N (nitrogen), P (phosphorus), and K (potassium) and secondary elements, such as Ca (calcium), Mg (magnesium), and S (sulfur). The micronutrients are B (boron), Cl (chlorine), Cu (copper), Fe (iron), Mn (manganese), Mo (molybdenum), Ni (nickel), and Zn (zinc) and are required in lower concentrations (Marschner, 2011).

### **Strategies for HLB management**

Given the lack of a cure for this disease, an integrated management must be implemented to reduce the symptoms and the death of trees infected with HLB. The said management consists of the following measures: determining how this disease enters the plant, in order to control the vector (Zhang *et al.*, 2014); establishing the temperatures at which the pathogen decreases (Fan *et al.*, 2016); finding out when the plant should be nourished to generate resistance (Velasco, 1999); and determining what product can be applied before and during the disease (Wang *et al.*, 2017).

### **International strategies for HLB management**

The Food and Agriculture Organization of the United Nations (FAO) and the United Nations Development Program (UNDP) developed strategies that were accepted as an international management for HLB (Aubert, 1990). This program consists of an integrated management: the control of psyllids with insecticide, the elimination of trees with HLB symptoms (to reduce the inoculum), geographical isolations, and nursery certifications (to propagate pathogen-free trees). To improve disease management, citrus phytosanitary agencies in Florida and Brazil require that tree production be carried out under insect-proof greenhouses, to enhance the management of the insect vector (Bassanezi *et al.*, 2011).

### **National HLB management strategies**

In Mexico, the strategies aimed at controlling HLB consist of a set of phytosanitary activities (Da-Graca and Kortsen, 2004) that involve producers, auxiliary plant health agencies, educational institutions, and research institutions (SENASICA, 2010). The tripartite strategies take place within the context of epidemiological surveillance. The first measure is the NOM-EM-047-FITO-2009 standard—which establishes phytosanitary actions to mitigate the risk of introduction and spread of HLB—; the second is eradication—which determines how to control the disease and the psyllid—; and the third is chemical and/or biological management (Mora-Aguilera, 2013).

## **CONCLUSIONS**

Given the current lack of established management for HLB, preventive measures must be taken, beginning using seedlings from certified nurseries, followed by soil and plant analysis that will help to determine an adequate and balanced nutrition and ultimately to generate resistance against this pathogen. The insect vector will not be able to generate resistance, if cultural, biological, and chemical management measures are applied in this precise order and there is no excessive use of chemicals.

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