

Identification of huanglongbing based on visual symptoms: a grower's diagnostic toolkit

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Abstract

Citrus Greening, which is mainly caused by bacteria, is one of the severe citrus diseases affecting all citrus cultivars and causing the deliberate abolition of trees worldwide. This infectious disease cannot be spread by wind, rain, or contact by contaminated personnel. The primary vector that

spreads this disease through feeding citrus leaves is the Asian citrus psyllid (ACP), a minuscule insect. The management of citrus greening is also very costly as there is no fruitful technique is developed to cure this disease except removing all infected plants from good ones to eliminate the dissemination of the pathogen. Citrus greening identification is also the most difficult job, as the symptoms are similar to other citrus diseases and nutrient deficiency. Asymmetrical blotchy mottling patterns on leaves are the main symptoms to detect this disease. Here we have discussed some visual signs of citrus greening, which will ultimately help root level farmers to identify and prevent this disease before it drastically impacts citrus plants. Whether it is affected by citrus greening or lack of nutrients, we have also discussed the pen test method of determining the symptoms as symmetrical or asymmetrical across the mid-vein.

Keywords: Huanglongbing, *Candidatus Liberibacter*, Asian citrus psyllid, blotchy mottle, visual symptoms

INTRODUCTION

Huanglongbing (HLB) disease is one of the greatest and ruinous diseases that drastically affect the citrus industry in many areas worldwide known as Citrus Greening, resulting in an unfinished ripening stage of fruits in affected trees, statistics show that it has been identified in more than 58 countries in the tropical and subtropical regions of Africa, Asia including India and Bangladesh (Tipu et al., 2017; Braswell et al., 2020). Surprisingly, the disease is called "Degeneration of Vein Phloem" (Tirtawidjaja et al., 1965). Citrus greening, among all citrus diseases, affects all citrus fruits (Bové, 2006; Gottwald, 2010; Wang et al., 2017). It is primarily a bacteria-induced disease that significantly reduces yield and exacerbates the economic value of citrus fruits, and ultimately dispatches the entire tree (Bové and Garnier, 2002; FAO, 2012; NASEM, 2018). This disease is

caused by the limited phloem, Gram-negative *Candidatus liberibacter* (α -Proteobacteria) bacteria, of which three (3) species are africanus, americanus, and asiaticus (Bové 2006; Wang and Trivedi 2013; Li et al., 2017; Rao et al., 2018), commonly known as (CLaf) (Jagoueix et al., 1994), (CLam) (Teixeira et al., 2005) (CLas) (Jagoueix et al., 1994). Inoculation begins during the feeding process involving two extremely mobile species of psyllids, *Diaphorina citri* Kuwayama (Asian citrus psyllid) for CLas and CLam, and *Trioza erytreae* Del Guercio (African citrus triozide) for CLaf (McClean et al., 1965; Capoor et al., 1967; Hall et al., 2013).

The spread of the disease is mainly associated with a minuscule insect called the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, which feeds primarily on citrus leaves (about 4 mm long). The main attraction for CLas and CLam species is young growing leaves (Halbert and Manjunath, 2004; Grafton-Cardwell et al., 2013; Hall et al., 2013), but it is obvious that those species exacerbate the vascular symptoms of the plant (Li et al., 2006; Tatineni et al., 2008; Li et al., 2009; Lopes et al., 2009; Coletta-Filho et al., 2014; Johnson et al., 2014). One study (Li et al., 2006) reported that the largest bacterial titers were recorded in the petioles and mid-ribs of the leaf, and the same bacterial populations were also observed in the peduncle, columella, and leaf midribs compared to seeds, young shoots, flower buds, flowers, and bark, which eventually opened a door to diagnose foliar tissue that was used as a common approach in previous approaches. One of the interesting facts about the Asian citrus psyllid (ACP) is that it can carry the bacterium for many days, and when feeding on an uninfected plant, it has the ability to transfer the bacterium. It takes just 30 minutes, however, to suck the bacteria from the infected leaves. (FAO, 2013; Molki et al., 2019). Recently, a few results showed that the host's root system was first destroyed by that bacterium as well as from the feeding location; it went through a process of multiplication in which it increased its population and then began its journey to leaves. (Johnson et al., 2014; Rao et al.,

2018). *Liberibacter* is one of the other bacteria (*Liberibacter*, *Serratia marcescens* (Zhang et al., 2005); *Candidatus Phytoplasma*, *Spiroplasma*, and *Candidatus Phlomobacter fragariae* (Zreik et al., 1998)) that could easily live within plant cells, mainly within phloem sieve cells.

Due to the movement of bacterium inside the roots and leaves, the phloem tissue of these two areas radically blocked, thus hindrance the movement of nutrients and sugars in the internal tissues (Bendix and Lewis, 2018), resulting in loss of leaves, uneven fruits size which can affect the taste and texture of fruits as well as premature fruit drop and the eventually the death of tree (FAO, 2013). In the midst of the grafting process, it could also be disseminated if the grafting tools have been taken from infected plant tissue (Grafton-Cardwell et al., 2006; Garcés-Giraldo, 2012; León and Kondo, 2017). In comparison with other infectious diseases, it cannot spread by wind or rain or contact by contaminated personnel. Greening has been recognized as a dreadful citrus disease that affects all cultivars of citrus and intentionally causes trees to be eliminated. In the citrus growing areas of the world, its insect vectors have been most observed, and this may occur due to gigantic movements of plants and insects around the globe (Tolba and Soliman, 2015) as well as some calamities such as hurricanes or storms have played an important role in expanding the Asian citrus psyllids over vast distances. (Gottwald 2010; Wang et al. 2017).

Among three *Candidatus Liberibacter* species, there is the fact that their ecological adaptation is not the same to each other considering temperature if we represent CLas, which fosters when the temperature is over 30°C, which manifested as a heat-tolerant (Hall et al., 2013) and the CLaf species are on the opposite which adaptation is closely associated when the temperature is below 30°C that's why in case of altitudinal distribution of the two pathogen species ecological preferences are first (Jagoueix et al., 1994). Citrus greening has drastically reduced its citrus production in a number of countries such as Asia (the indigenous citrus home), Africa, the Indian

subcontinent, the Arabian Peninsula, and a handful of islands in the Indian Ocean, and also in American territories (Spann et al., 2008; Tipu et al., 2020).

A valid reason of losing the commercial value of fruits affected by HLB along with the yield reduction, falling market price, management of disease and production is very costly, which severely affects the economic condition and also citrus greening highly deteriorate the marketability of fruits in agricultural field (Inoue et al., 2020). When a tree begins to be infected, it is totally a loss project to apply chemical pesticide because it cannot cure completely with pesticides; in addition, young plants are more susceptible to this disease, and it is therefore a very laborious job for farmers to replace the young plants that have been affected so far. Recently, no fruitful method has been developed to cure this deadliest disease, except to remove all infected plants from good ones to eliminate the spread of the pathogen (Inoue et al., 2020). One of the most difficult tasks is to identify citrus greening and other disorders due to the likeliness between HLB and other citrus diseases such as Citrus tristeza virus and problems with nutrient deficiency, which are often confusing. (McClellan & Schwarz, 1970; Albrecht et al., 2016).

It is difficult to conclude a correct diagnosis at the onset of the disease. In comparison with warmer months, HLB symptoms in cooler months are more apparent, McCollum and Baldwin (2017) noted. One interesting fact is that for several months or years without manifesting any symptoms (a symptomless phase), HLB infected plants could bear huge bacteria, which is an important factor for infecting other uninfected plants. Although symptoms can be found throughout the year, but most likely from September to March, there has been a highly accelerated symptomatic visibility of that disease. Symptoms are easily seen on shade or overcast days, as well as almost all parts of the plants, including the canopy, leaves, twigs, and fruit where infection occurs; the entire tree decreases rapidly as the disease progresses.

LEAF SYMPTOMS

Blotchy mottling patterns on the leaves are asymmetrical using the mid-rib as a central line, which is by far the inevitable diagnostic greening symptom, and it is between said as a severe infection and incidentally similar to the sign of Fe deficiency when leaves turn into discolored yellow (Inoue et al., 2020) (Fig. 1). Nevertheless, the symmetrical pattern would be observed if plants suffer from nutrients and other disorders (i.e., almost exact replicas of each half using the mid-rib as a mirror). One study (Ammar et al., 2013) found that a large number of nymphs were found on both sides of the leaf, approximately 64.5 percent and 35.5 percent on the lower (abaxial) side of the leaf and the upper (adaxial) side of the leaf, respectively, where more numbers were found on the lower side of the leaf than on the upper leaf. Usually, plants show these nutrient deficiencies and blotchy mottle that are affected by HLB; however, these symptoms are not definitive HLB symptoms, and the blotchy mottle is mainly present in young or mature leaves anywhere in the tree canopy. For greening, vein corking and yellow veins alone are not diagnostic. If present, however, check the tree canopy for blotchy mottle symptoms as well. Mid-vein, 'corky' mid-veins/ under-rib, interveinal chlorosis, bright yellow shoots among a green canopy, pronounced/prominent.

FRUIT SYMPTOMS

As an infected tree deteriorates rapidly, the fruit may also begin to display signs of disease. The color of the stylus end shows the yellow/orange color when healthy fruits reach their maturity, and the green color is associated with the peduncle end. While fruits are affected by HLB disease, on the other hand, the peduncular end is yellow/orange and the stylus end is still green (Bové, 2014) (Fig. 2). Lopsided, oblong, misshapen, or small green fruit may be the external outlook of the fruit.

Inversion of fruit color formation (fruit yellowing from top to down on citrus varieties of orange color. A few numbers of sour orange are tolerant among all varieties, and their symptoms are not as dreadful as compared to others. Fruit dropping is started in its early stage of life due to having symptomatic fruits in infected trees, which means immature fruits are falling off, and sub-sequencing (Dala-Paula et al. 2019).

Although a number of these symptoms can be confused with deficiencies and disorders, the symptoms usually manifest in one branch or part of the tree at first, then slowly spread throughout the entire canopy, while deficiencies and disorders within the canopy and among trees are usually more uniform.

WHOLE TREE SYMPTOMS

In narrow upright leaves, as well as in yellow shoots, the blotch mottle and yellow veins are seen (Fig. 3). In addition, a number of symptoms, including shoot dieback, stunting, off-season bloom, overall yellow appearance, and drop in fruit, have been recorded. Symptoms were only seen on one part of the canopy now and then. It has been a common incident that the root system is particularly destroyed by CLas infected trees; thus, when associated with HLB disease, a large number of root losses occur (Graham et al., 2013). Before the really visible symptoms appear, initiation of root dieback observed, the proportion of root-shoot ratio decreases over time in HLB affected trees (Johnson et al., 2014). One thing is clarified by (Shahzad et al., 2020) that health tress is a higher amount of biomass for root, shoot, and lead compared to the HLB affected trees, and data revealed remarkably that when affected with HLB, nearly 40 percent to 50 percent less root biomass is recorded compared to healthy trees.

DIFFERENCES BETWEEN HLB AND OTHER NUTRIENT DEFICIENCY PROBLEMS

Diagnosing the mineral nutrient deficiency from greening symptoms is very crucial because it is also responsible for leaf yellowing due to the absence of few nutrients like zinc, iron, manganese, and calcium resulting from the mineral deficiencies, which also similar to greening symptoms. Diffuse asymmetric mottle is produced when HLB affects the plant, which can be shown as atypical patches of light green or yellow in contrast to the normal green color of the leaf. It has been a confusing phenomenon to distinguish between HLB symptoms and nutrient deficiency symptoms, but it could be isolated by a cautious and precise identification. At the later stage of the disease, nutrient deficiency symptoms are often exposed, and for each symptom, it will have different patterns, but one thing is fixed that patterns always occur across the mid-vein.

Zinc (Zn) deficiency: In each leaf, an interveinal yellow mottle is observed which is symmetrical with respect to the central vein, but at the time of extreme deficiency, the leaves are often small, narrow, and chlorotic; other than that, HLB could also occur (Fig. 4A).

Manganese (Mn) deficiency: It is almost similar to zinc deficiency, but the combination of colors, which is between light green and dark green, is a bit different; young leaves, however, have this kind of deficiency (Fig. 4B).

Magnesium (Mg) deficiency: In the event of this deficiency, an upturned "V" form was manifested, green patches of color on both sides of the central vein differentiating from the yellow of the remaining leaves, and aged leaves appear to be more affected than others (Fig. 4C).

Iron (Fe) deficiency: Normally, the leaves with light green and young, as well as which are almost pale those are affected by iron deficiency (Fig. 4D).

Calcium (Ca) deficiency: A deficiency of calcium in citrus is expressed as a fading of the chlorophyll along the leaf margins and between the main veins during the winter months. Small

necrotic (dead) spots can develop in the faded areas. Calcium deficiency produces small, thickened leaves and causes loss of vigor, thinning of foliage and decreased fruit production (Fig. 4E).

Nitrogen deficiency: Due to nitrogen deficiency, the whole leaf color turns into light yellow, and the size of the leaf is reduced (Fig. 4F).

HOW TO DETECT GREENING

It is always the trickiest job to identify greening disease in a grower's orchard, especially when trees are facing impoverished health. One of the essential symptoms to diagnose a greening disease is blotchy mottle, and it has been recognized as a typical symptom by which anyone could detect the disease, but numerous studies revealed that during the month from June to August (summer months), it is quite tough to pinpoint the disease as that time trees are actively raising; therefore it comes out that ones have a best chance to distinguish this particular disease from the month of September to May (fall, winter, and spring) (Spann et al., 2008). There is no fixed phenomenon about where leaf symptoms normally occur; it may observe anywhere on the tree; for this reason, the branch is affected with diseased leaf needs to be taken off, and it is consequential to check inside the tree. When the spread of the disease is severe in the tree, it is suggested to a quick checkup whether there are any symptoms or not in the fruits. It is a great approach to locate the specific infected tree by making a visible spot on the particular branch for sorting out among the healthy and unhealthy trees.

IDENTIFICATION OF SYMPTOMS OF CITRUS GREENING AT FIELD LEVEL

It has been studied that the expressive symptoms of HLB disease occur in several parts of the trees, including most importantly in leaf, fruit, twigs, and branches. However, it is always found confusing to detect that disease as it is expressed some variable symptoms, which is tough to understand at grower's level without knowing their patterns precisely as well as some symptoms may match with other citrus diseases. In this paragraph, we have tried to come up with some important and ambiguous symptoms explicitly so that it will immensely assist root level farmers to take necessary steps to identify and prevent these diseases before these diseases drastically ruin the whole disease. Here, we focused on some crucial visual symptoms; in Fig. 5A, blotchy mottle present does not resemble on both sides of the mid-vein, which is a principal symptom, as well as leaf notching symptoms, are recorded due to intense psyllid feeding. In the case of Fig. 5B, psyllid nymphs and adults are seen at the lower portion of leaf and twigs, but the size of nymphs is too tiny. Moreover, now and then mid vein radically exposed with yellow color from top to bottom, apart from that a standard symptom like a green island on yellow leaf manifested in some foliage (Fig. 5C), when it comes to fruit, the size of the fruits is lessened, misshapen and lopsided fruit (Fig. 5D), in addition, the taste unveiling bitter and salty than the normal one.

PEN TEST

This test is well known as a visual diagnosis test, which precisely measures the homogenous patterns on both surfaces of the leaf, with the help of this method of determining the symptoms as symmetrical or asymmetrical across the mid-vein, whether it is HLB-affected or lacks nutrient. (Vashisth et al., 2016). To conduct this, we need to mark on the leaf by making two circles on antipodal halves, which should be represented at the central vein beside one another as shown in Fig. 6; therefore, with the help of this, it is more convenient to detect greening symptoms or not

such as, if the circles represent the identical symptoms then it must be symmetrical which indicate no sign of greening. But naturally, nutrient deficiency symptoms show an exactly alike pattern in both circles, apart from that HLB blotchy mottle pattern don't resemble the areas of two circles; thus, it concludes that it has greening symptoms.

CONCLUSION

It is difficult, sometimes not affordable, for growers to detect citrus greening by using molecular tools. This diagnostic guideline will definitely be an easy toolkit for detecting huanglongbing in citrus orchard with limited technical knowledge. However, this review emphasizes on field diagnosis by visual investigation, additional detection though molecular techniques is necessary to study and confirm the pathogen.

AUTHOR CONTRIBUTION

MMHT was responsible for the conceptualization, overall write up of the review manuscript. MMHT, MMM worked for figures interpretation. RJ, AB, AKMAH helped to edit and finalization of the draft manuscript. AB helped to writing summary and MMHT wrote conclusion of the manuscript.

DECLARATION OF COMPETING INTEREST

All authors are wish to confirm that there is no potential conflict of interest.

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Figure(s)

Fig. 1. Diagnostic blotchy mottle leaf pattern of HLB (A), vein corking (B), green islands (C), and blotchy mottle (D) in leaves.



Fig. 2. Huanglongbing infected orange trees bear small and lopsided fruits (right) relative to healthy fruits (left) (A) (Credit: Tom Benitez), Lopsided fruit (B), and Misshapen fruit (C).



Fig. 3. An HLB-affected tree in which the symptomatic leaves (A,B), shoot dieback (C), and Excessive fruit drop (D).

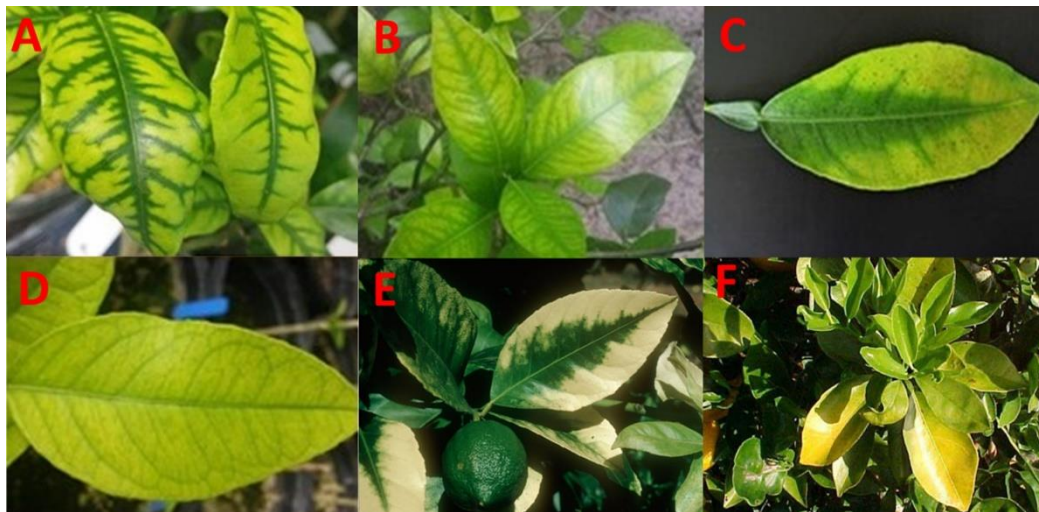


Fig. 4. Different nutrient deficiency symptoms, e.g. Zinc deficiency (A), Manganese (Mn) deficiency (B), Magnesium deficiency (C), Iron deficiency (D), Calcium deficiency (E), and Nitrogen deficiency (F).

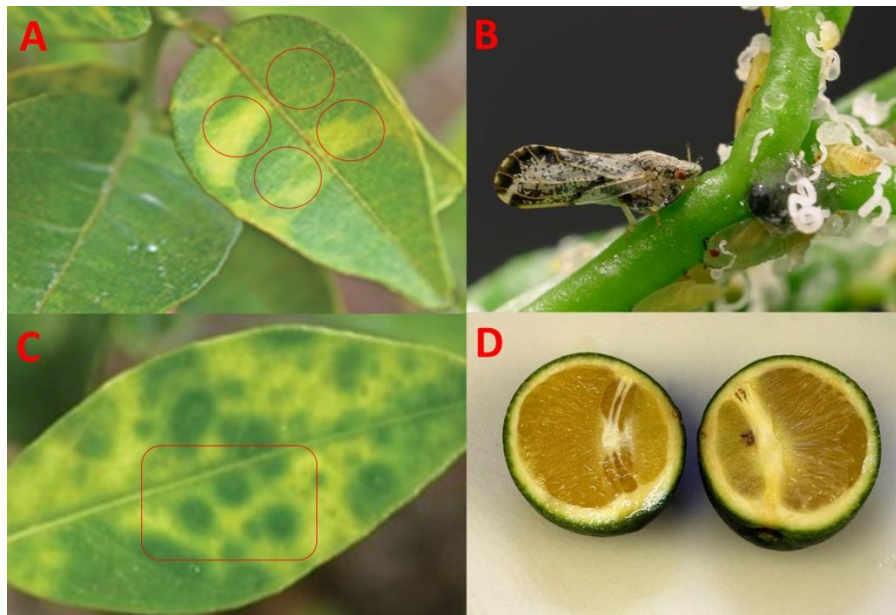


Fig. 5. Diagnostic symptoms/ signs of huanglongbing at field level showing dissimilar blotchy mottle pattern on both sides (A), presence of citrus psyllid nymph and adult (B), green Island like view on the leaf surface (C), and lopsided fruit with aborted seeds (D).

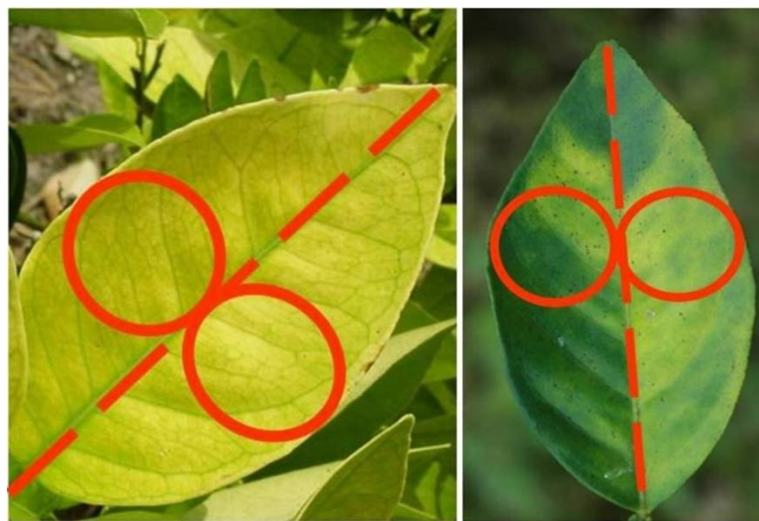


Fig. 6. Nutrient deficiency (left) compared to HLB symptoms (right); notice the symmetry on either side of the midvein in the nutrient-deficient leaf compared to the symptoms' asymmetrical pattern HLB affected leaf.