

# VISUAL DIAGNOSTIC OF CITRUS “HUANGLONGBING” DISEASE

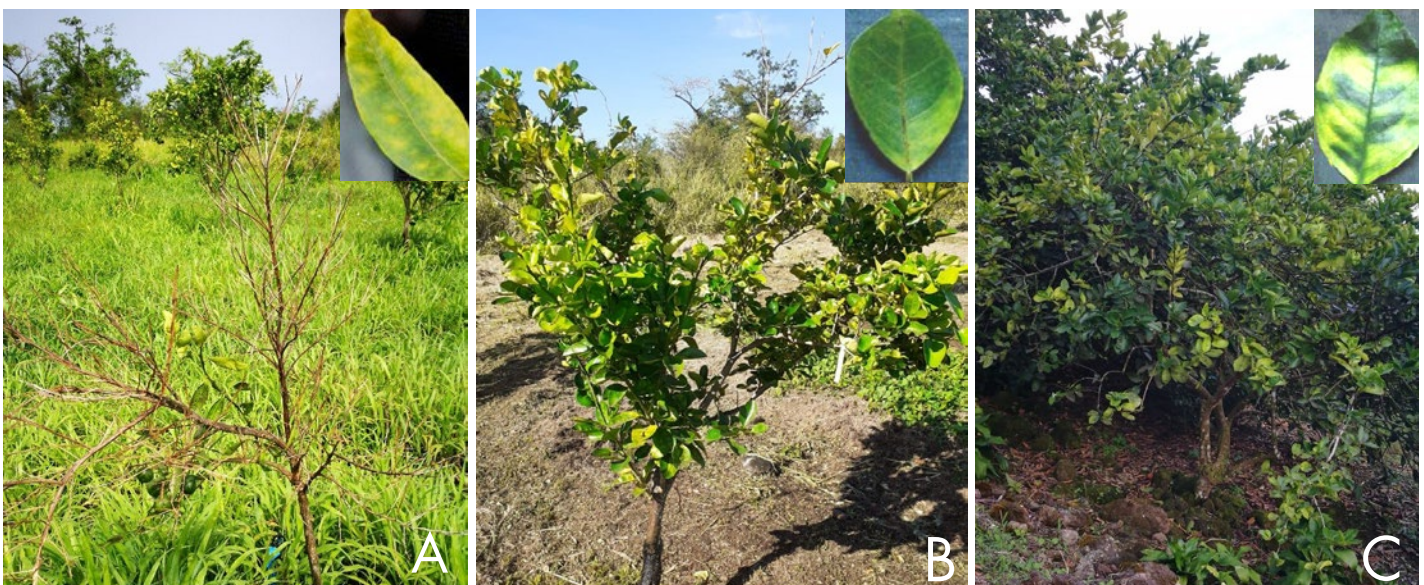
How to recognize different types of symptoms in infected trees, leaves and fruits



## ■ THE PROBLEM ADDRESSED

### “Huanglongbing”: a lethal disease of citrus

Citrus is the most important fruit tree crop in the world, with an annual production estimated in 165 million tonnes (FAOSTAT, 2019). “Huanglongbing” (HLB) is considered the most destructive disease of commercial citrus species worldwide. It affects all citrus varieties, severely reducing the yield and the performance of the crops. The disease is associated with the presence of ‘*Candidatus Liberibacter*’ species and it is transmitted by the psyllids *Diaphorina citri* and *Trioza erytreae*. To date, HLB is present in every continent except Australia and mainland Europe, although one of its insect vectors was detected in the Iberian Peninsula in 2014 (Cocuzza *et al.*, 2017). Its impact is very high in the Americas and Africa. For example, in Florida, HLB caused losses of 4,554 million US dollars in only six years (2005-2011) (Hodges and Spreen, 2012). Since there is no effective control except to prevent trees from becoming infected, awareness of the disease and rapid identification of its symptoms are essential. However, there is still a clear lack of information about “huanglongbing”, especially in those countries that are still free of the disease.



- Infected citrus plots in Guadeloupe: A, mandarins; B, oranges; C, lime

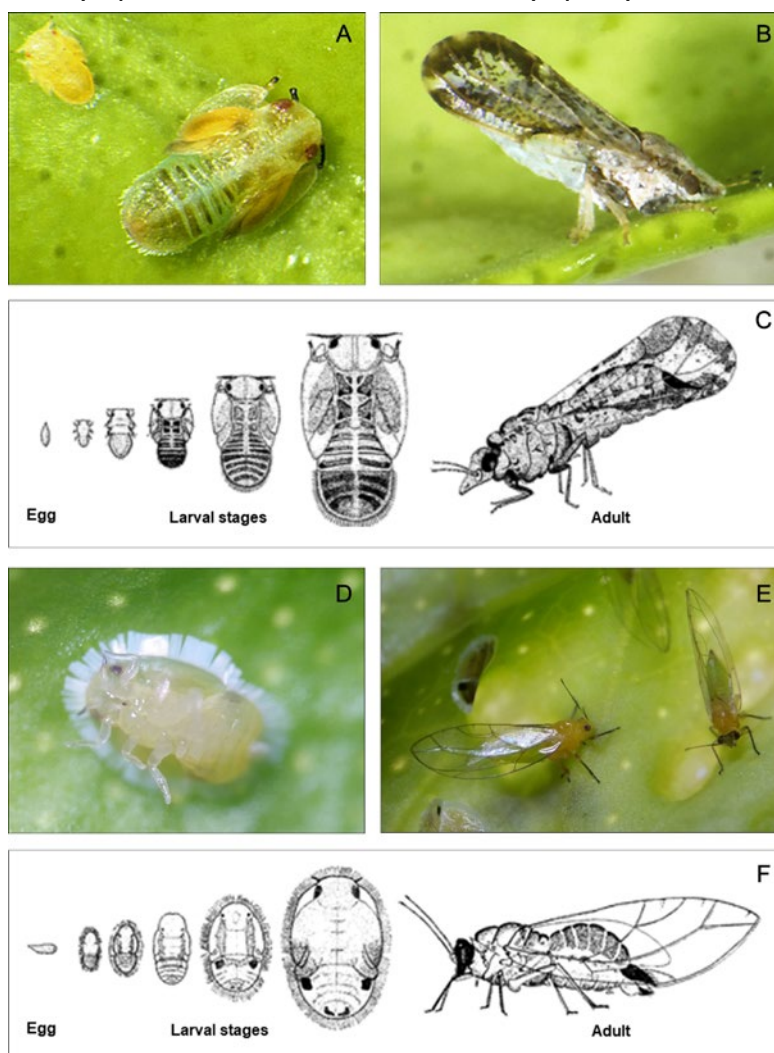
## ■ THE PRACTICE/INNOVATION PROPOSED BY TROPICSAFE

### Insect vectors and symptoms of the HLB disease

The ‘*Candidatus Liberibacter*’ responsible of citrus “huanglongbing” disease slow the plant growth, reduce flowering and generate losses of production in quantity and quality, before eventually the tree dies (Fujikawa *et al.*, 2013). Two species of citrus psyllids can transmit this bacterium: *Diaphorina citri* (Asian citrus psyllid) and *Trioza erytreae* (African citrus psyllid). The inoculation period is about one hour and the symptoms appear about four months after the infection (Batool *et al.*, 2007). *D. citri* is present in most tropical regions in Asia and America and also in all the citrus producing areas in the Caribbean. This psyllid is the most dangerous and widespread species in the world. *T. erytreae* is present in Africa, the Canary Islands, Madeira and recently in north of Spain and Portugal. There is a major concern that the arrival of these psyllids would have a disastrous effect in Europe (ANSES, 2019). The first step for disease prevention is to detect the disease and its insect vectors presence. It is necessary to detect as early as possible the presence of the vector and the disease in citrus orchards and in private gardens, in order to establish the appropriate HLB management practices. Protocols for monitoring the vectors and detecting the disease are part



of the TROPICSAFE project. It was found that very small quantities of psyllids are sufficient to infect orchards, with sometimes non-symptomatic trees that can die off very quickly.



- Nymph (A) and adult (B) of *Diaphorina citri* (CIRAD), instars of *D. citri* (C). Nymph (D) and adult (E) of *Trioza erytreae*, instars of *T. erytreae* (F) (A. Tena, IVIA)

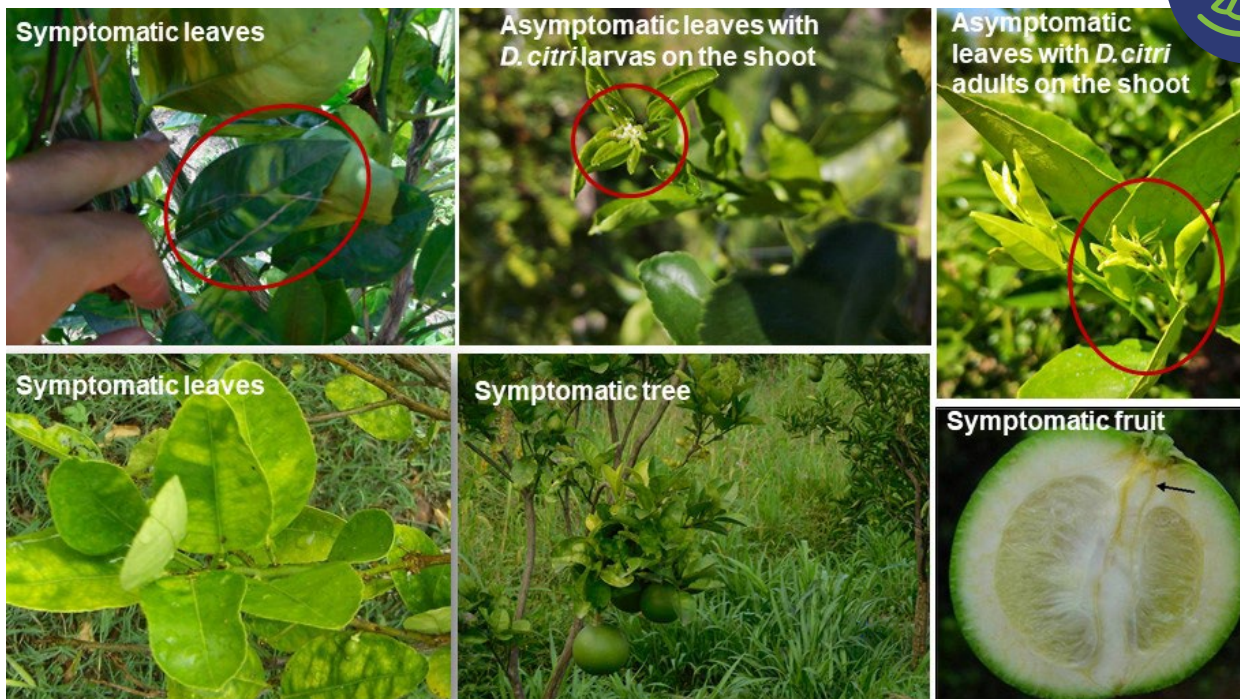
## ■ HOW IS TROPICSAFE IMPLEMENTING IT?

### How to detect insect vectors and HLB in the field

Both insect vectors are identifiable in both nymph and adult stages by visual and/or microscopic observations.

Symptoms of HLB are characteristic and can be easily recognized in the field: asymmetric yellow blotch of leaves and desiccation, premature fall, deformation of fruits. Leaves may also become thicker, leathery, the midribs and the lateral veins are sometimes enlarged, swollen and corky (Batool *et al.*, 2007). However, some trees can stay asymptomatic at the beginning of the disease, therefore, a molecular detection of '*Candidatus liberibacter*' is necessary.

Different DNA amplification methods, including polymerase chain reaction (PCR), quantitative PCR, nested PCR, and loop-mediated isothermal amplification (LAMP), can be used to detect HLB in the plant samples (Iftikhar *et al.*, 2016). However, these laboratory-based detection methods are often time-consuming and expensive. TROPICSAFE project is now developing more practical and accessible detection techniques that could be performed in the field.



• Major symptoms of HLB on citrus plants, leaves and fruits (ASSOFWI)

■ HOW IS IT WORKING?

## Detection of the HLB in a small scale survey in the project countries

Symptomatic citrus samples were collected in Cuba, Jamaica, and Guadeloupe. DNA extraction was performed from 1 g of leaf midribs. PCR amplification for 'Candidatus Liberibacter' species was performed with primers OA1/OI2 and OI1/OI2c (Jagouveix *et al.*, 1996), the sequencing confirmed the presence of 'Candidatus Liberibacter asiaticus' in the majority of the tested samples. The presence of the pathogen resulted not related to the tree variety or to the geographic locations.

Sample	Location	HLB positive/total tested
<b>Cuba</b>		
Persian lime	Ceballos/Ciego de Avila	21/21
Orange Valencia		30/30
Tangerine		1/1
Grapefruit		12/12
Persian lime	Sola/Camagüey	12/12
Orange Valencia		2/2
Grapefruit		2/2
Persian lime	Jagüey/Matanzas	15/16
Orange		29/30
Tangerine		2/2
Grapefruit		2/2
Lemon		3/3
Orange	Arimao/Cienfuegos	2/2
Grapefruit		6/6
Mexican lime	La Habana	0/1



Sample	Location	HLB positive/total tested	
<b>Guadeloupe</b>			
Orange Valencia Rod Red	Trois-Rivières	0/1	
Orange Navelina		0/1	
Mandarine Creole		1/1	
Tahiti lime		4/4	
Mexican lime	Nord Vieux-Habitants	1/1	
Tahiti lime		1/1	
Tangelo Nova	Vieux-Habitants	0/1	
Tangelo Jackson		1/2	
Orange Navel		1/1	
Mandarine Tample		2/2	
Orange Valencia Late		1/2	
Orange Maltaise		0/1	
Mandarine Falglo		1/1	
Tangelo Triumph		1/1	
Tangor Ellendale		1/1	
Orange Navelate		1/1	
Orange Fisher Navel		1/2	
Flhor AG1 4X		CIRAD Capesterre	0/2
<b>Jamaica</b>			
Citrus company	Bay Brook	4/5	
Citrus Montego Bay	Montego Bay	1/1	

- Results of the survey for the detection of '*Candidatus Liberibacter*' in citrus species in Cuba, Guadeloupe and Jamaica (Bertaccini *et al.*, 2019)

#### KEY WORDS

Detection, symptomatology, citrus, disease, insect vectors

#### FURTHER INFORMATION

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