Tropical Plant Pathology (2020) 45:279–284
https://doi.org/10.1007/s40858-020-00363-8

REVIEW

The status of citrus Huanglongbing in China

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Received: 31 January 2020 / Accepted: 28 April 2020 / Published online: 24 June 2020

Abstract
The Chinese citrus industry is facing an unprecedented challenge from citrus Huanglongbing (HLB). As no resistant commercial citrus varieties and no curable chemicals are available so far, it is still the "no. 1 threat" to Chinese citrus industry. This review article outlines the current status of the disease prevalence and measures recommended and adopted for the prevention and control of HLB in China. Research progress in many areas are highlighted together with the challenges and future prospects of HLB in China. A few practical suggestions have been made based on my analysis.

Keywords Citrus Huanglongbing · Prevention & control · Research progress · China

Introduction

Citrus Huanglongbing (HLB) was first reported to have occurred in Taiwan (Sawada 1913) and Guangdong (Reinking 1919), with a history of more than a century in China. Zhao (1981) reviewed the status of HLB in China by then, and a detailed review booklet on research and control of HLB was published (Zhao 2017). As no resistant commercial citrus varieties and no curable chemicals are available so far, it is still the "no. 1 threat" to Chinese citrus industry.

Current epidemic trends

HLB has occurred in over 300 counties of 10 provinces in the mainland of China, including Guangdong, Guangxi, Fujian, Zhejiang, Jiangxi, Hunan, Yunnan, Guizhou, Hainan and Sichuan, and has destroyed millions of mu (15 mu = 1 ha) of Chinese citrus orchards in history. In particular, citrus production in Guangdong, Guangxi and Fujian has been disturbed by the disease for a long time. Since 2013, there has been an outbreak of HLB in Ganzhou, Jiangxi province. About 50 million diseased trees have been destroyed so far, but more than 2/3 of the citrus industry in this area have been saved after managed for 6 years. At present, this navel orange-produced region is in a low epidemic state (Fig.1).

In recent years, the citrus industry has developed fast in Guangxi, Hunan, Jiangxi, Sichuan, Chongqing, Hubei provinces. Based on four citrus superior belts, the changes of citrus production are calculated for three zoning regions. As shown in Fig. 2, the region production of Zhejiang-Fujian-Guangdong provinces declined mainly due to the increase of labor/land cost and HLB problem, whereas that of the other two regions of Guangxi-Hunan-Jiangxi and Sichuan-Chongqing-Hubei provinces increased. Combined with the crucial task for poverty alleviation, the development enthusiasm of superior navel orange belt of south Jiangxi-south Hunan-north Guangxi has been keeping in a high status with impressive returns for recent years, especially Guangxi, causing the production of citrus nursery trees out of control with hidden risk. In order to prevent even large-scale outbreak of HLB, the government of Guangxi province has released an Act for effective regulations on prevention and control of HLB since November 2019, which is the first provincial Act with this specific issue in China.

Meanwhile, its vector Asian citrus psyllids (*Diaphorina citri*, ACP) continue to move north, and have arrived in Jinsha river valley located at Pingshan county of Yibin prefecture, Sichuan province, and several counties near the south part of Xuefeng mountain of Hunan province. Therefore, the Yangtze river citrus superior belt and western Hubei-western Hunan mandarin superior belt are facing the challenge of HLB invasion, the prevention and control situation is still grim.

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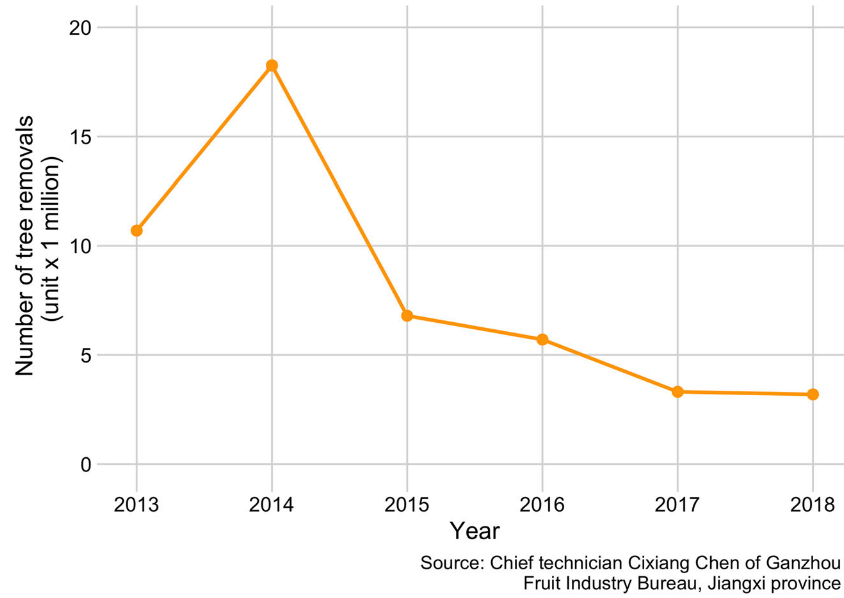

Fig. 1 Number of citrus tree removals due to the attack of Huanglongbing in Ganzhou, Jiangxi province, China

Current status of prevention and control

In 2014–2018, the Ministry of Agriculture and Rural Affairs (MARA) of P.R. China had annually convened a national on-site conference on HLB prevention and control in Guilin (2014), Ganzhou (2015–2017) and Guangzhou (2018), respectively. The attention paid by the MARA is unprecedented! A national HLB census was also organized, formulating the HLB diagnostic criteria, prevention and control norms, releasing disaster relief funds, and setting up a state key research program *etc.* In 2017, the "National collaborative innovation alliance for integrated prevention and control of HLB for citrus superior belts" was established, so as to strengthen the collaborative linkage throughout the country, effectively curbing the HLB harm. There are many success cases of prevention and control based on "Three basic measures" (application of HLB-free nursery trees, timely removal of HLB-infected trees and large-scale joint prevention and control of ACP), other useful practices include "village rules and people's covenants", "technician-driven management", "reduced HLB trees reward", "highly dense planting", "windbreaks", "killing summer shoots via chemicals" *etc.*, effectively curbed the epidemic situation of the disease.

For the safety of Yangtze river citrus superior belt and western Hubei-western Hunan mandarin superior belt, the MARA and the government of Sichuan province have been building up HLB-intercepted zone at Jinsha river valley of Pingshan county, Yibin prefecture, Sichuan province since 2017, the MARA combined with the government of Hunan and Guangxi provinces, is in the process of construction of another HLB-intercepted zone at Xuefeng mountain area, mainly in Xinning county, Shaoyang prefecture, Hunan province (Fig. 3).

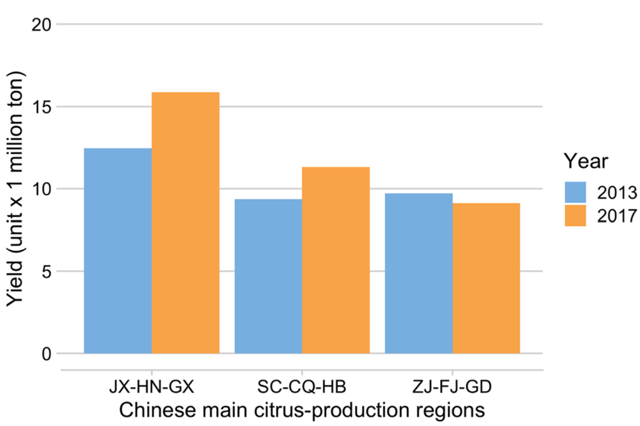

Fig. 2 The citrus yields of three Chinese dominant regions in 2013 and 2017. SC-CQ-HB: Sichuan-Chongqing-Hubei provinces; JX-HN-GX: Guangxi-Hunan-Jiangxi provinces; ZJ-FJ-GD: Zhejiang-Fujian-Guangdong provinces

Research progress

Since the causal pathogen (*Candidatus* Liberibacter asiaticus, *C*Las) of HLB in China has not been successfully cultured *in vitro*, the in depth research on its physical and chemical characteristics and the development of pathogen control technology has still been in a difficult situation.

Dagnostics Progress before 2018 was summarized by Zheng and collaborators. Since then, there have been a few publications: Lou et al. (2018) developed a tandem repeat-based polymerase chain displacement reaction with similar sensitivity to real-time PCR to detect ca 5000 samples by now in Guangxi province. Qian et al. (2018) and Zhong et al. (2018) modified recombinase polymerase amplification and

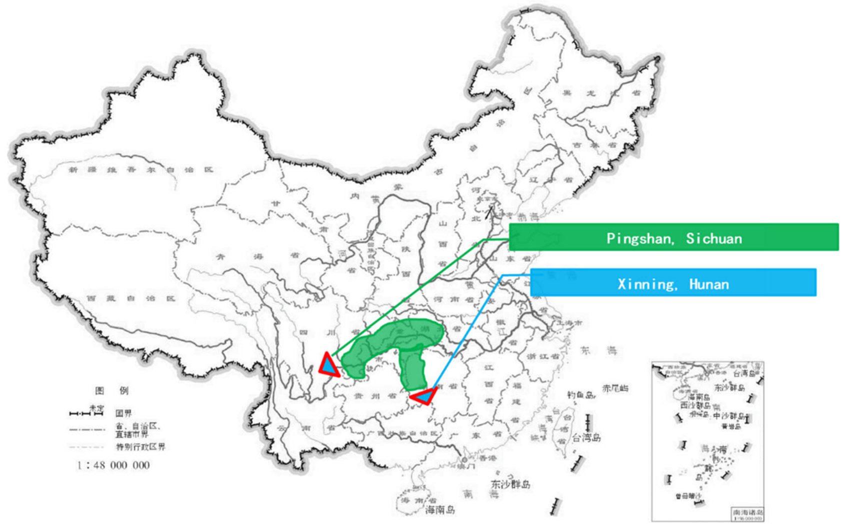

Fig. 3 The schematic representation of two citrus Huanglongbing-intercepted zones constructed/planned in China

droplet digital PCR in China, respectively, which have proved to be easier to operate with higher sensitivity, but have not approached for large scale of field practice so far. Bao et al. (2019) utilized a rectified primer OI1 by adding a G base missed before to enhance PCR capacity of detecting *C*Las, ca 1000 samples have been addressed by this method by now in Guangdong province. Other addressed techniques for non-destructive fast detection of *C*Las, include Raman spectroscopy technology combined with partial least squares discriminant analysis (Liu et al. 2018a, b; Weng et al. 2018), direct ionization mass spectrometry (Li et al. 2019a, b), near infrared spectrum (Meng et al. 2019a, b, c), UAV hyperspectral remote sensing (Lan et al. 2019), contractive auto-encoder combined extreme learning machine and stacked denoising auto-encoders (Lu et al. 2019a, b), however, they are still in the research stage, especially the accuracy needs to be improved, and have not been applied to field practical detection. Detecting strip, developed by antibodies against several expressed proteins derived from *C*Las, is still on the way, as the sensitivity is not yet quite satisfactory for field practice. At the moment, different laboratories use their own preferable diagnostic methods to detect *C*Las in China, although a national standard one is setting up to follow.

CLas and its interaction with citrus Zheng et al. (2018a, b) found a new prophage named Type 3, which is not capable of reproduction *via* the lytic cycle, may be useful against Type 1 prophage-phage invasion. Fu et al. (2019a, b, c) reported that two major prophage typing groups were associated with two altitude-dependent origins of *C*Las in China, and *C*Las preferentially colonizes adjacent phloem cells in a vertical rather than horizontal direction in citrus, and mild strains of *Citrus tristeza virus* may provide some protection against *C*Las by limiting its multiplication and spread. Peng et al. (2019) reported that the callose genes *CsCalS5*, *CsCalS7* and *CsCalS8* were up-regulated in *C*Las-affected Shatangju'(*Citrus reticulata*) and Chandler pummelo (*C. grandis*). Wen et al. (2019) reported that the expression of phloem protein CsPP2B15 in the young leaf tissue of Jincheng sweet orange (*C. sinensis*) was significantly induced by *C*Las-infecton, while it was repressed in sour pommelo. Deng et al. (2018) reported that the lower levels of phloem disruption, together with greater phloem regeneration, are two key elements that contribute to HLB tolerance in diverse citrus cultivars. Hu et al. (2018a, b, c) found that starch metabolism and photosynthesis of *C. hystrix* were not affected after *C*Las-infection. Hu et al. (2018a, b, c) found that trunk injection of salicylic acid, acibenzolar-S-methyl and antibiotics was effective, and the first two significantly induced the expression of PR-1 and PR-2, while potassium phosphate and oxalic acid significantly induced the expression of PR-2 and PR-15. Liu et al. (2019a, b) reported that CLIBASIA_00460, one of the *C*Las-encoded secretion-dependent presecretory proteins, may be a novel virulence factor of *C*Las, and the nuclear localization of this protein was temperature dependent and positively correlated with its pathogenicity in planta. The above research progress has not reached the field application status. Currently, quite a few laboratories in China have focused on seeking useful effectors secreted by *C*Las to interact with citrus, which might offer some light of hope to control HLB in the future.

Asian citrus psyllids (ACP) Progress before 2018 can refer to the review (Yao et al. 2018). Zhao et al. (2018) demonstrated that yellow is the best trapping color, followed by blue, whereas Li et al. (2019a, b) reported that ACP is sensitive to ultraviolet, blue and green light. Acquisition efficiency of *C*Las by ACP was highest in nymphs reared at 25 °C on a host plant with high *C*Las titers (Wu et al. 2018a, b). The concentration of *Wolbachia* was highest in 4th and 5th instar nymphs of ACP (Ren et al. 2018). There were significant differences in endophytes of different ACP stages, but they were mainly *Proteobacteria*, and its concentration was high at 2nd-5th instar nymphs of ACP, while 1st instar nymphs could acquire *C*Las (Meng et al. 2019a, b, c). In Ganzhou, prophages of *C*las within ACP were mainly Types A and B, and few Type C (Chen et al. 2018). DcitOBP1 protein might play a crucial role in host-plant volatile odorants perception in ACP (Wang et al. 2019). Zhong et al. (2019) found that Chongyi wild mandarin (*C. reticulata*) attracted more ACP, as it has more secondary metabolites flavonoids, less carotenoids and more salicylic acid than jasmonic acid. Wu et al. (2018a, b) reported that the mitochondrial sequences of ACP were divided into three types: south China type, south Asia type, Pakistan/American type, and their origin migration was predicted. Mitochondrial DNA of ACP was found to have basically no host differentiation (Meng et al. 2018). Neuropeptides and their receptors in ACP were preliminarily studied (Wang et al. 2018). Zhang et al. (2019) proposed that ACP invaded China from 2 routes with atmospheric changes and recent human activities, and then spread from 3 routes in China. Lu et al. (2019) found that the chitin synthase gene of ACP was highly expressed in the body wall and in 5th instar nymphs. It was also found that ACP had strong resistance to imidacloprid and chlorpyrifos, which was associated with the expression of cytochrome genes CYP4C68 and CYP4G70 (Tian et al. 2018). Several DcitABC transporters were highly expressed in fat body, midgut and the hindgut with Malpighian tubules, in particular, DcitABCG11 and DcitABCG14 were most highly expressed in the hindgut, the transcript abundance of 11 DcitABC genes was significantly upregulated upon exposure to a LC50 concentration of imidacloprid, and the expressions of three cytochrome P450 genes and one glutathione S-transferase gene were also significantly elevated (Liu et al. 2019a, b). The predictive model for threshold control of ACP was established, $R0 < 1$ could be prevented and controlled, otherwise it could not be controlled (Tu et al. 2019). The above research progress is most limited to laboratory stage so far, although yellow trap boards are commonly used in field in China, the control of psyllids mainly relies on chemical measure, sometimes also with the aid of windbreaks.

Prevention and control Progress before 2018 can refer to the reviews (Zhou 2018; Munir et al. 2018; Sang et al. 2018). The emulsifiable concentrate formulation containing *Sophora alopecuroides* extract might be developed as an eco-friendly novel prophylactic against ACP (Rizvi et al. 2019). It was found that the mixing effect of carotenoids: limonene: linalool (3:3:1) was significantly better (Song et al. 2019). *Cordyceps javanica* GZQ-1 isolated from ACP adults was highly pathogenic to ACP nymphs and adults (Ou et al. 2019). *Serratia marcescens* KH-001 isolated from ACP nymphs could effectively kill 83% of ACP nymphs, and its fermentation efficacy was reduced to 40% (Hu et al. 2018a, b, c). Among the 39 antimicrobials (non-antibiotics), 21 were ineffective and 10 were significantly enhanced (Yang et al. 2018). Clothianidin application through a drip irrigation system exhibits excellent efficacy against ACP (Meng et al. 2019a, b, c). Dinotefuran and cyantranil are the best pesticides sprayed on foliage, whereas thiamethoxam was the most effective pesticide applied to soil (Cheng et al. 2019). Yao et al. (2019) reported that both *C*Las titer and antibiotics gradually decreased within 90 d post injection (dpi) of oxytetracycline (OTC), AGP enzyme expression decreased most after injection of 0.2 g/tree, while starch degradation was not obvious after injection of 0.1 g/tree. Some of the above findings could have potential of field application, further field trials with medium scale are still on the way. Although the biological control measure is very much in demand for eco-friendly goal, it has not been largely practiced in the field for controlling HLB in China.

Challenges and prospects

Firstly, the government has to face a few realistic difficulties, such as property rights disputes with orchard owners in the implementation of measure "timely removal of HLB-infected trees", organization hardship in the execution of "large-scale joint prevention and control of ACP ", unplanned problem in nursery trees production, leading to difficulty in implementing regional defense from spreading. This has led some experts, managers and farmers to question the vitality of the "Three basic measures". Some scientists even proposed that "since it is the quarantine object, it should be completely eliminated!". This point of view is unrealistic, because once psyllids are introduced into an area and established the population, it is virtually impossible to eliminate them, and the realistic practice is to control HLB prevalence to less than 3%. In other words, double wheels driven both by science/technology and administration are in realistic need!

Secondly, the delay in construction of efficient monitoring and early warning system of HLB and ACP has affected the speed of timely removal of diseased trees and invasion of ACP. Thirdly, the in depth research on pathogen-vector-host interactions is relatively lagging behind, which has affected the development of eco-friendly prevention and control measures. Fourthly, prevention awareness is crucial, and taking the right prevention and control measures is even more important! Fertilizer correction or sawing diseased branches has proved to be great harmful, many orchards and even the entire industry are thus delayed effective prevention and control opportunities! Practice has proved that “Three basic measures” is still the most effective strategy for HLB control, unless the biology of HLB pathogen has made a breakthrough! Many research activities are still being carried out through national and provincial projects.

The citrus industry in China’s coastal areas has been more or less disturbed by HLB for a long term, its northward movement has been causing increased losses in some regions, which has exerted a huge impact on the citrus industrial economy at county- or prefecture-level once it outbreaks. As China’s total citrus production capacity reached about 40 million tons, of which only ca 1 million tons for export within the recent decade, therefore, due to the adjustment of domestic market price effect, the overall economic loss caused by HLB for the whole Chinese citrus industry will be at a relatively low level. After the fast development of Chinese citrus industry, the risk of HLB outbreaks still exists in some counties or prefectures. It is still very much in demand of constructing efficient monitoring and early warning system by using IT means, and strengthening basic research based on above problems. It is suggested that the government further explores to establish negative case propaganda, to guide professional cooperation, and enterprises act as the main body of prevention and control, complementing “village rules and people’s covenants”, “technician-driven management” experiences, guiding classified management, so as to enrich “Three basic measures” system.

Acknowledgements This work was partially supported by the National Key R&D Program of China (2018YFD0201500) and the National Natural Science Foundation of China (31671992).

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