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Quantification and ecological study of 'Candidatus Liberibacter asiaticus' in citrus hosts, rootstocks and the Asian citrus psyllid

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The use of proper management strategies for citrus huanglongbing (HLB), caused by 'Candidatus Liberibacter asiaticus' (Las) and transmitted by Asian citrus psyllid (ACP) (Diaphorina citri), is a priority issue. HLB control is based on healthy seedlings, tolerant rootstock cultivars and reduction of ACP populations. Here, dynamic populations of Las in different citrus hosts and each instar of ACP were studied, together with the seasonal growth and distribution of Las in different tissues, using conventional and TaqMan real-time PCR. Different levels of susceptibility/tolerance to HLB were seen, resulting in different degrees of symptom severity and growth effects on hosts or rootstocks. Troyer citrange, Swingle citrumelo and wood apple were highly tolerant among 11 rootstock cultivars. Regarding distribution and seasonal analysis of Las, mature and old leaves contained high concentrations in cool temperatures in autumn and spring. Las was detected earlier through psyllid transmission than through graft inoculation, and the amounts of Las (AOL) varied in different hosts. Thus, different AOL (10^4–10^7 copy numbers μL^{-1}) and Las-carrying percentages (LCP; 40–53.3%) were observed in each citrus cultivar and on psyllids, respectively. Furthermore, both AOL and LCP were lower in nymphs than in adult psyllids, whereas the LCP of psyllids were not affected by increasing the acquisition-access time. The present study has significant implications for disease ecology. The combination of early detection, use of suitable rootstocks and constraint of psyllid populations could achieve better management of HLB disease.

Keywords: 'Candidatus Liberibacter asiaticus', acquisition-access time, Asian citrus psyllid, dynamic population, huanglongbing, susceptibility/tolerance

Introduction

Citrus huanglongbing (HLB), also known as greening, is currently the most destructive disease in most citrus planting areas worldwide. Currently, three nonculturable phloem-limited Gram-negative a-proteobacteria are reported associated with HLB disease: 'Candidatus Liberibacter asiaticus' (Las) from Asia, 'Candidatus Liberibacter africanus' (Laf) from Africa and 'Candidatus Liberibacter americanus' (Lam) from South America (Garnier et al., 2000; Bové, 2006). The Asian citrus psyllid (ACP), Diaphorina citri (Hemiptera: Liviidae), is the main insect vector of 'Candidatus Liberibacter' spp. in Asia and America, whereas the African psyllid, Trioza erytreae (Triozidae), is the main vector in Africa (Aubert, 1987; Halbert & Manjunath, 2004). The causal agent can infect all commercially important citrus cultivars, resulting in major reductions in fruit quality and yield and the appearance of foliar symptoms, such as irregular mottling and severe chlorosis with nutritional deficiency-like syndrome (McClean & Schwarz, 1970), followed by incomplete colouring of mature fruit (da Graça, 1991) and shortening of the lifespan of infected trees (Miyakawa, 1980). HLB is spread by vegetative propagation and insect vectors, and thus far, there is no cure for this severe disease. HLB has been the most important factor limiting citrus production worldwide.

Because there are no cures for HLB disease, prevention and vector control are critical for HLB integrated management. It is proposed that two important strategies for prevention are sensitive detection methods and a healthy seedling system. In recent years, quantitative detection methods have been developed and improved for more sensitive and specific purposes (Li et al., 2006, 2009; Morgan et al., 2012; Ananthakrishnan et al., 2013; Hu et al., 2013; Bertolini et al., 2014; Kogenaru et al., 2014; Feng et al., 2015). Quantitative methods have been used to analyse the distribution of Las, showing uneven patterns in different infected

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Table 3 Effect of huanglongbing (HLB) on the growth of commercial citrus and citrus rootstock cultivars in Taiwan

	Tree[a]	Original height (cm)	Average height in 1 year (cm)	Growth rate in 1 year (cm)	Disease index[b]
Citrus cultivar					
Ponkan mandarin	D	25	43.75	18.75	S
	H		85	60	H
Tonkan mandarin	D		39.5	14.5	S
	H		73	48	H
Valencia sweet orange	D		55.25	30.25	S
	H		90	75	H
Wentan pomelo	D		47	22	I
	H		87	62	H
Eureka lemon	D		88.75	63.75	I
	H		116	91	H
Kumquat	D		43.5	18.5	I
	H		57	32	H
Rootstock cultivar					
Sunki mandarin	D	50	68	18	S
	H		118	68	H
Calamondin	D		68.25	18.25	S
	H		122	72	H
Bitter orange	D		62.5	12.5	S
	H		110	60	H
Volkamer lemon	D		94	44	I
	H		125	75	H
Rough lemon	D		86	36	I
	H		120	70	H
Rangpur lime	D		95.75	45.75	I
	H		128	78	H
Alemow	D		98.5	48.5	I
	H		128	78	H
Chinese box orange	D		78	28	I
	H		103	53	H
Troyer citrange	D		102.25	52.25	M
	H		137	87	H
Swingle citrumelo	D		87.25	37.25	M
	H		118	68	H
Wood apple	D	40	52.5	32.5	H
	H		80	40	H

[a]H, healthy control; D, diseased.
[b]Disease index: S, severe; I, intermediate; M, mild; H, healthy.

Valencia sweet orange, 49 days in Eureka and 56 days in Wentan (Fig. 4a). The replication rates of Las in each cultivar showed similar trends. Las replicated more easily and faster in Ponkan and Valencia sweet orange than in Eureka and Wentan. In the first 2 months after graft inoculation, the replication rate of Las was faster in Valencia sweet orange than in Ponkan, but Las was detected earlier in Ponkan. The amounts of Las were nearly 10^4 copy numbers in Valencia sweet orange (2.34×10^4) and Ponkan (9.48×10^3) at 63 and 77 days after inoculation, respectively. The amounts of Las reached the highest level (1.9×10^7) at 154 days after inoculation. In contrast, the amounts of Las remained low ($\sim 10^3$) in Eureka and Wentan during the first 7 weeks and increased up to 4.86×10^3 copy numbers at 5 months after graft inoculation. Symptoms were observed in Ponkan and Valencia sweet orange at 4–5 months after inoculation. The upper leaves of Ponkan showed vein yellowing, and small, hard and yellowing symptoms were observed on the young leaves at 6 months after inoculation. Similar symptoms were observed on the upper leaves of Valencia sweet orange and obvious vein yellowing was easily observed on new upper young leaves. In contrast, no obvious symptoms were observed in Eureka and Wentan at 6 months after inoculation (Fig. 5).

Las was detected earlier through psyllid transmission compared to graft inoculation at 21 days after infection (Fig. 4b). However, Las replicated slowly in Ponkan and Valencia sweet orange through psyllid transmission. The amounts of Las in Ponkan reached 1.33×10^3 copy numbers at 8 weeks after infection, whereas hundreds to thousands of copies of Las remained in Valencia sweet orange. At 4 months after infection on Ponkan, only the new young leaves on the ends of shoots showed small, hard leaves with vein yellowing symptoms. No obvious symptoms were observed on infected Valencia sweet orange (Fig. 5).

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Figure 3 Growth effects of 11 rootstock cultivars infected with 'Candidatus Liberibacter asiaticus'. SK, Sunki mandarin; Cal, calamondin; KT, bitter orange; Vol, Volkamer lemon; RgL, rough lemon; RL, Rangpur lime; TC, Troyer citrange; SW, Swingle citrumelo; Mac, alemow; Sev, Chinese box orange; and WA, wood apple. The left plant of each pair is the healthy control. Photographs were taken 1 year after infection. [Colour figure can be viewed at wileyonlinelibrary.com]

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Table 4 Distribution and seasonal population dynamics of 'Candidatus Liberibacter asiaticus' (Las) bacteria in three citrus species, Hong-Jian sweet orange, Murcott tangor and Peiyu pomelo

Season	Position	PCR index[a]		
		Hong-Jian	Murcott	Peiyu
Spring	New flush	±	±	–
	Young leaf	+	++	±
	Midrib of mature leaf	+++	+++	+++
	Mesophyll of mature leaf	++	++	++
	Midrib of old leaf	+++	nt	nt
	Fruit	+++	++ (flower)	+ (flower)
	Bark of young twig	+++	+++	++
	Bark of trunk	–	–	–
	Root	++	+	+
Summer	New flush	–	–	–
	Young leaf	±	±	±
	Midrib of mature leaf	+++	++	+++
	Mesophyll of mature leaf	++	++	++
	Midrib of old leaf	+++	nt	nt
	Fruit	+	+++	+++
	Bark of young twig	++	++	++
	Bark of trunk	–	–	–
	Root	+	+	+
Autumn	New flush	±	±	±
	Young leaf	++	++	+
	Midrib of mature leaf	+++	+++	+++
	Mesophyll of mature leaf	++	++	++
	Midrib of old leaf	+++	nt	nt
	Fruit	+++	+++	+++
	Bark of young twig	+++	+++	+++
	Bark of trunk	–	–	–
	Root	+	+	++
Winter	New flush	nt	nt	nt
	Young leaf	±	+	±
	Midrib of mature leaf	+++	+++	++
	Mesophyll of mature leaf	++	++	++
	Midrib of old leaf	+++	nt	nt
	Fruit	+++	+++	+++
	Bark of young twig	+++	+++	+++
	Bark of trunk	–	–	–
	Root	+	++	++

nt, not tested.

[a]PCR index: density count of the Las-specific band on agarose gel measured by a densitometer. Pixel value –, ≤14; ±, 15–49, +, 50–109; ++, 110–169; +++, 170–230. All values were determined as the average of four individual plants.

Quantitative detection of Las in citrus cultivars and Las-carrying percentages on psyllid populations in the field

The highest amounts of Las were detected in Murcott tangor (2.15×10^7), followed by Ponkan mandarin (2.42×10^6), Valencia sweet orange (1.06×10^6), calamondin (9.33×10^5), and Wentan pomelo (4.18×10^4; Table 5). These data indicated that each citrus cultivar had different responses to Las infection, resulting in varying levels of symptoms and proliferation. The average percentages of Las-carrying psyllids from the three infected fields ranged from 40% to 53.3% (Table 5).

Quantitative detection of Las and Las-carrying percentages on different instars of nymphs of psyllid

No Las was detected in the instars of egg and 1st nymph stages (Table 6). Tens and hundreds of copies of Las were detected in individual nymphs from the 2nd to 5th instars. The average percentage of Las-carrying instars of nymphs was 33.9%. These results indicated that the amounts of Las and Las-carrying individuals were both lower in nymphs than in adult psyllids.

Test of acquisition-access of Las in psyllids

The results of Las acquisition tests are summarized in Table 7. The amounts of Las (hundreds to thousands of copy numbers) had no obvious fluctuations within psyllids during the acquisition-access time. The percentages of Las-carrying psyllids were 40–60%, indicating that the increasing acquisition-access time did not affect the percentages.

Discussion

HLB, the main devastating and systemic disease on citrus worldwide, is caused by Las in most citrus planting areas. Thus far, strategies for the control of HLB rely on prevention methods, such as using tolerant rootstock cultivars, implementing a healthy seedling system, eliminating infected plants and intermediate hosts in the field and preventing insect vectors. However, the lack of knowledge regarding disease ecology and pathogen transmission might leave blind spots when establishing control methods. In the present study, the susceptibility/tolerance to HLB of commercially important citrus and rootstock cultivars was determined and the dynamic population of Las in citrus hosts or psyllids was demonstrated using conventional PCR (Hung et al., 1999) and TaqMan real-time PCR (Feng et al., 2015). The TaqMan real-time PCR developed here showed high specificity and sensitivity to the amounts of Las in citrus plants or even in one or a few psyllids. The present study provided more information on HLB based on the application of a quantitative detection method for tracking the dynamic proliferation of Las in citrus and psyllids.

Susceptibility/tolerance testing was carried out on the main commercial citrus and common rootstock cultivars in Taiwan. The determinations were based on the replication rates of Las, symptom severity and the growth rate on infected plants. Measures were taken to ensure that all experimental plants avoided any viral interference. Similar results between Las-infected plants in the

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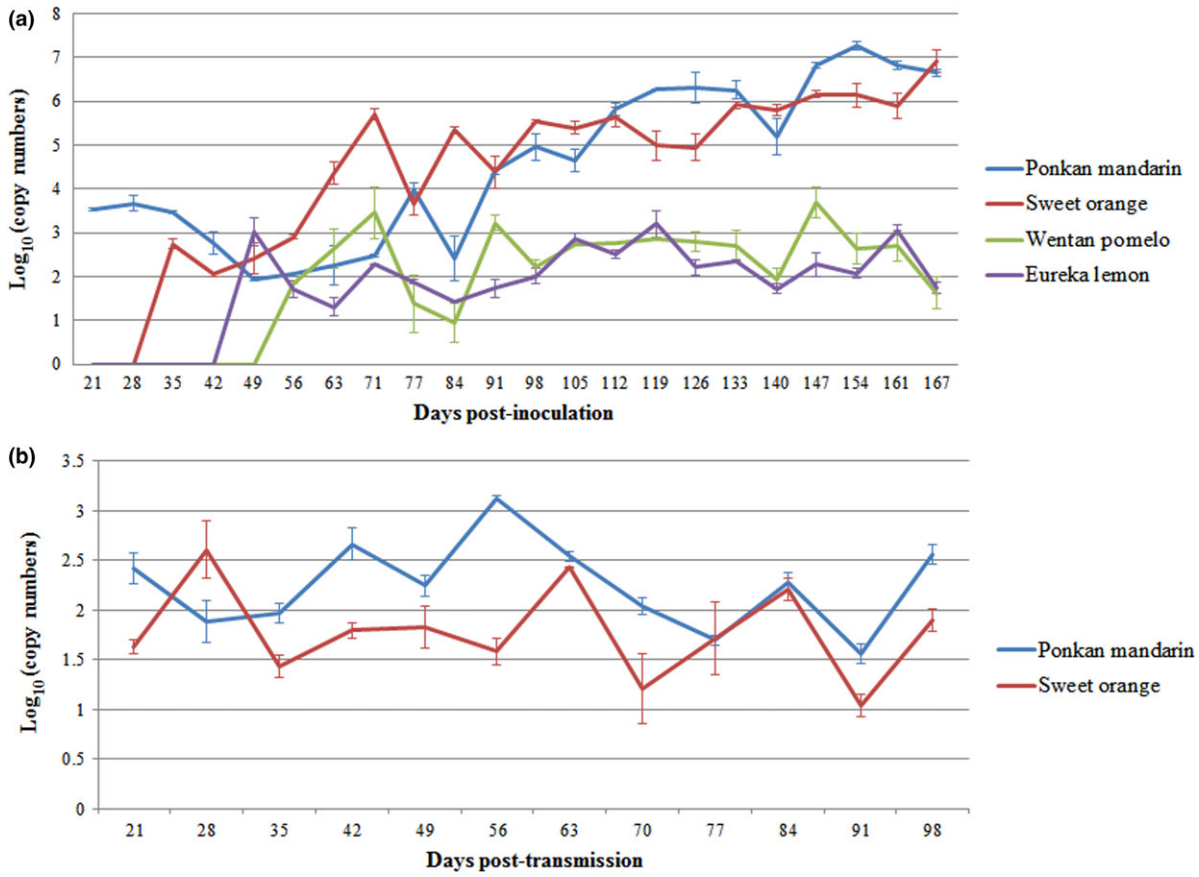

Figure 4 Quantitative detection using real-time PCR of the dynamic replication of 'Candidatus Liberibacter asiaticus' (Las) in (a) four Las-inoculated citrus cultivars (Ponkan mandarin, Valencia sweet orange, Wentan pomelo and Eureka lemon) after graft inoculation, and (b) two citrus cultivars (Ponkan mandarin and Valencia sweet orange) after psyllid transmission. [Colour figure can be viewed at wileyonlinelibrary.com]

laboratory or field showed that Ponkan and Tankan mandarins are susceptible to Las and are unable to grow after infection. Valencia sweet orange is less susceptible, whereas Wentan pomelo and Eureka lemon are tolerant to Las and are able to grow slowly. Kumquat is more tolerant but is still infected through inoculation. The present study confirmed that Las strain II, isolated from the field in a previous study, showed high virulence and multiplied quickly in all commercially important citrus cultivars in Taiwan (Tsai et al., 2008). The present study also illustrated a positive relationship between HLB symptom severity on citrus leaves and the proliferation rate of Las using PCR detection (Coletta-Filho et al., 2010). In addition, more susceptible cultivars had much higher concentrations of Las. Based on the present pathological study, more molecular evidence is needed to elucidate the factors involved in different levels of susceptibility/tolerance of various cultivars while facing Las infection. It is assumed that different citrus cultivars might show differing gene expressions or responses against Las infection. Previous studies have also shown that many metabolites were detected at higher concentrations in the tolerant cultivars and might play roles in conferring tolerance to HLB (Albrecht et al., 2016).

The analysis of the amounts of Las in different tissues and the seasonal population dynamics in host plants showed that Las was unevenly distributed in different tissues (Tatineni et al., 2008; Li et al., 2009). The effect of live or dead populations of Las was not considered in this study. According to Hu et al. (2014), the population trends between live and dead Las were similar (although with different titres) and so it was assumed that it would show little effect on the results. The highest amounts of Las were detected in the transportation tissues of fruit in every citrus cultivar. These results might explain the typical symptoms of Las-infected fruits, such as greening and thick skin, resulting in poor quality fruits. In contrast, no Las was detected in the bark of the stem base of citrus, suggesting that Las might not accumulate in the main transportation region of the stem base. It is assumed that new flush retained good growth potential and that Las moved too slowly to invade newly growing leaves. The uneven distribution of Las in different tissues might be because nutrition-rich propagated or metabolic tissues such as fruit, young and mature leaves are beneficial to Las proliferation and accumulation. In addition, previous studies analysing the dynamic population of Las revealed that the Las population decreased in the spring (Wang

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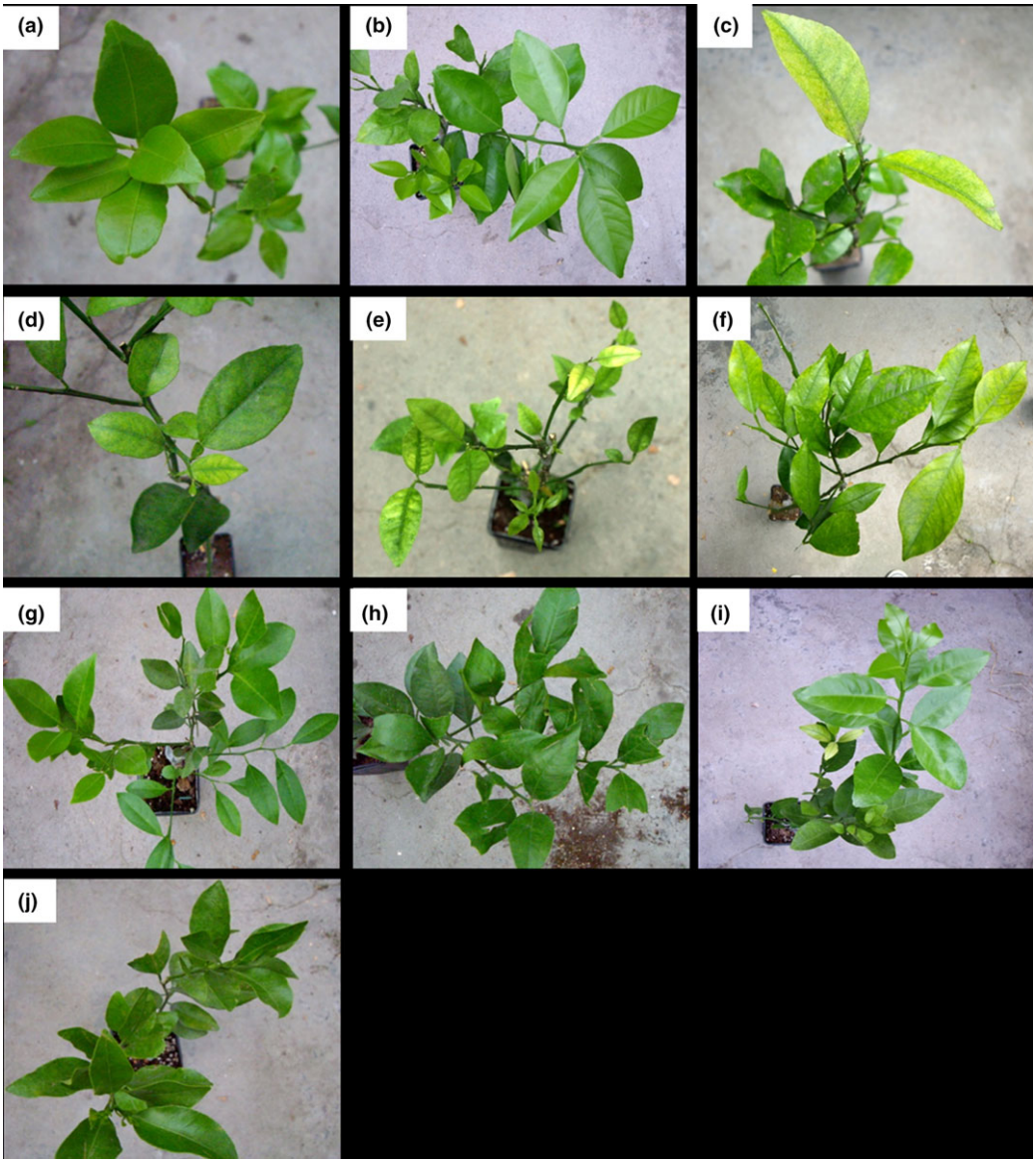

Figure 5 Symptom development on Ponkan mandarin (PM) and Valencia sweet orange (SO) infected with 'Candidatus Liberibacter asiaticus' (Las) after graft inoculation and psyllid transmission. (a) PM before graft inoculation; (b) PM 3 months after graft inoculation; (c) PM 6 months after graft inoculation; (d) SO before graft inoculation; (e) SO 3 months after graft inoculation; (f) SO 6 months after graft inoculation; (g), PM 2 months after psyllid transmission; (h) PM 4 months after psyllid transmission; (i) SO 2 months after psyllid transmission; and (j) SO 4 months after psyllid transmission. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 5 Quantitative detection of 'Candidatus Liberibacter asiaticus' (Las) in different Las-infected citrus cultivars and determination of Las-carrying percentage on psyllid populations in fields by real-time PCR

Citrus cultivar	Source	Average of Las quantities in citrus (copies)	Average of Las quantities in psyllid (copies)[a]	Las-infected psyllids (%)
Ponkan mandarin	Chuchi village	2.42×10^6	3.41×10^5	42.9
Murcott tangor	Chiayi city	2.15×10^7	4.25×10^2	40.0
Valencia sweet orange	Chuchi village	1.06×10^6	1.98×10^4	53.3
Wentan pomelo	Chuchi village	4.18×10^4	–	–
Calamondin	Taipei city	9.33×10^5	–	–

[a]7, 5 and 38 psyllids were collected from Ponkan, Murcott and Valencia cultivars, respectively. No psyllids were found on Wentan or calamondin in the field survey. Mean copy numbers were obtained with triplicate assays for each sample extract by real-time PCR.

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Table 6 Quantitative determination of 'Candidatus Liberibacter asiaticus' (Las) and Las-carrying percentage on different instars of psyllid nymphs collected from Las-infected sweet oranges in fields by real-time PCR

Instar	Numbers of psyllid tested	Average of Las quantities in psyllid (copies)	Las-infected psyllid (%)
Egg	10	0	0.0
1st	15	0	0.0
2nd	10	6.5	10.0
3rd	9	42.8	22.2
4th	6	331.1	66.7
5th	19	33.3	36.8

et al., 2006) or winter (Hu et al., 2014), which differed from the data obtained in the present study. This finding suggests that different sampling methods and environments might affect investigations of the Las population. Generally, Las was easily detected in new flush in the spring and autumn rather than in the summer. This result agrees with Hung (2006), who illustrated the wide spread of HLB disease during the spring was because the highest populations of psyllid were observed in the sprouting period of Murcott tangor in the spring. Psyllids migrated and transferred Las much more frequently during this period time.

Previous studies have indicated that different citrus cultivars have different susceptibility or tolerance levels to HLB disease (Garnier et al., 1984; Bové & Garnier, 2002). Similar results showed that Ponkan and Valencia sweet orange were more susceptible to HLB and Las using PCR at 3 and 5 weeks after grafting, respectively. In contrast, Las was detected at 7 and 8 weeks after grafting in Eureka and Wentan, respectively. Real-time PCR showed greater sensitivity for detecting Las earlier in these citrus hosts than conventional PCR, and Las was also amplified at different rates in each host. Although the uneven distribution of Las in different tissues while sampling (Huang, 1987) might lead to unreliable detection, the long-term detection still showed an obvious trend in Las amplification. Compared with the Las-grafting method, psyllid transmission resulted in faster Las amplification in citrus hosts during early infection, but the amounts of Las were lower after 3 months. The difference between grafting and psyllid inoculations might reflect different pathways of Las infection. Las could directly enter sieve tube cells and subsequently replicate via the stylet of the psyllid, whereas graft-inoculated Las could be slower to move in to the host after the graft heals. The amounts of Las in infected scions were 100 times greater than those in psyllids, indicating the different replication rates between these two vectors.

The results of the Las acquisition test in psyllids, which were similar to a previous study (Hung, 2006), indicated that continuing to acquire Las day after day did not obviously enhance the amounts or the Las-carrying percentages in psyllids. Even if healthy psyllids were forced to acquire Las on infected plants for an extended

Table 7 Quantitative detection of 'Candidatus Liberibacter asiaticus' (Las) in psyllids individually with different acquisition-access time by real-time PCR

	Acquisition time (days)													
Psyllid	2	4	6	8	10	12	14	16	18	20	22	24	26	28
	Amount of Las [log(copy numbers)]													
1	0	0	2.32	0	2.27	2.84	0	2.21	0	0	0	2.00	1.65	3.14
2	2.40	0	2.58	2.13	1.87	2.16	0	0	0	0	0	2.09	0	0
3	0	2.33	0	0	0	0	0	2.17	0	0	0	1.83	0	2.58
4	2.22	0	3.42	0	0	0	0	0	2.46	2.58	1.90	0	0	0
5	2.35	3.01	1.55	0	0	0	2.16	0	2.52	2.76	1.89	0	2.61	0
6	0	3.21	1.88	0	0	2.26	0	2.16	0	2.23	3.13	0	0	2.07
7	2.16	2.75	0	2.27	1.78	0	2.01	0	0	2.60	1.84	1.82	1.63	2.42
8	0	0	0	2.04	0	1.90	2.27	2.56	2.11	2.87	0	1.89	1.44	0
9	0	0	0	0	2.10	0	2.33	0	3.54	0	1.97	2.15	1.63	2.48
10	0	0	0	2.36	2.16	0	2.41	1.58	0	0	0	0	0	0
Mean	2.29	2.93	2.82	2.22	2.07	2.34	2.26	2.24	3.02	2.66	2.52	1.98	2.05	2.69
Psyllids with Las (%)	40	40	50	40	50	40	50	50	40	50	50	60	50	50

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period, only a portion of the population would successfully acquire Las (Manjunath et al., 2008). In contrast, the amounts of Las inside psyllids collected from infected fields were hundreds and thousands of times greater than in the psyllids from the acquisition test. However, the Las-carrying percentage of psyllids from the field also remained near 40–53.3%, showing results similar to those of psyllids from the acquisition test. According to real-time PCR detection, only 5.71% of these Las-carrying psyllids had Las copy numbers in the thousands. This phenomenon might be related to the feeding habits of psyllids. Similar evidence was also reported by Pelz-Stelinski et al. (2010). Ammar et al. (2017) demonstrated that the salivary glands and midgut of psyllids may act as transmission barriers that can impede translocation of Las within the vector, and this evidence could explain the low Las infection rate in vector psyllid. In addition, the results of detecting psyllid nymphs on infected citrus plants in the field using PCR revealed that nymphs (2nd to 5th instars) could carry Las, consistent with a previous study (Hung et al., 2004). Furthermore, it was observed that the amounts of Las were lower in nymphs than in adult psyllids, indicating that Las could continually replicate during psyllid growth. These results were similar to those of a previous study showing high replication rates of Las in the nymph stages of psyllids (Ammar et al., 2016b). It is concluded that most psyllids carried Las at the instar nymph stage and that Las replicates to high concentrations in adult instars, showing the capacity to transmit Las in the fields.

The present study evaluated the levels of susceptibility of different citrus and rootstock cultivars to HLB, which might help the establishment of healthy seedling systems to select proper tolerant cultivars as rootstocks. Hung (2006) showed that only one Las-carrying psyllid could transmit Las to infect citrus plants. However, only 12.9% of psyllids could successfully infect plants, and the amounts of Las in each psyllid were significantly different, consistent with the results of a previous study (Ukuda-Hosokawa et al., 2015). The data also showed that a low proportion of nymph or adult psyllids in the population carried high amounts of Las, suggesting that the successful Las transmission rate was associated with the amounts of Las in each psyllid and the numbers of psyllid populations. Monitoring and decreasing the psyllid population numbers could effectively reduce the chance of Las-carrying individuals infecting citrus plants. This study, based on quantitative methods, is relevant not only to research on Las pathogenicity in citrus hosts or in psyllid vectors but also to efforts to enhance the current understanding of HLB disease ecology.

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References

Albrecht U, Fiehn O, Bowman KD, 2016. Metabolic variations in different citrus rootstock cultivars associated with different responses to Huanglongbing. Plant Physiology and Biochemistry 107, 33–44.

Ammar ED, Ramos JE, Hall DG, 2016. Acquisition, replication and inoculation of Candidatus Liberibacter asiaticus following various acquisition periods on huanglongbing-infected citrus by nymphs and adults of the Asian Citrus Psyllid. PLoS ONE 11, e0159594.

Ammar ED, Hall DG, Shatters RG, 2017. Ultrastructure of the salivary glands, alimentary canal and bacteria-like organisms in the Asian citrus psyllid, vector of citrus huanglongbing disease bacteria. Journal of Microscopy and Ultrastructure 5, 9–20.

Ananthakrishnan G, Choudhary N, Roy A et al., 2013. Development of primers and probes for genus and species specific detection of 'Candidatus Liberibacter species' by real-time PCR. Plant Disease 97, 1235–43.

Aubert B, 1987. Trioza erytreae Del Guercio and Diaphorina citri Kuwayama (Homoptera: Psylloidea), the two vectors of citrus greening disease: biological aspects and possible control strategies. Fruit 42, 149–62.

Bertolini E, Felipe RTA, Sauer AV et al., 2014. Tissue-print and squash real-time PCR for direct detection of 'Candidatus Liberibacter' species in citrus plants and psyllid vectors. Plant Pathology 63, 1149–58.

Bové JM, 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. Journal of Plant Pathology 88, 7–37.

Bové JM, Garnier M, 2002. Phloem and xylem restricted plant pathogenic bacteria. Plant Science 163, 1083–98.

Coletta-Filho HD, Carlos EF, Alves KCS et al., 2010. In planta multiplication and graft transmission of 'Candidatus Liberibacter asiaticus' revealed by real-time PCR. European Journal of Plant Pathology 126, 53–60.

Feng YC, Tsai CH, Vung S, 2015. Cochin China atalantia (Atalantia citroides) as a new alternative host of the bacterium causing citrus Huanglongbing. Australasian Plant Pathology 44, 71.

Garnier M, Danel N, Bové JM, 1984. The greening organism is a Gram-negative bacterium. In: Garnsey SM, ed. Proceedings of the 9th Conference of the International Organization of Citrus Virologists. IOCV: University of California Riverside, USA, 115–24.

Garnier M, Jagoueix-Eveillard S, Cronje PR, 2000. Genomic characterization of a liberibacter present in an ornamental rutaceous tree, Calodendrum capense, in the Western Cape province of South Africa. Proposal of 'Candidatus Liberibacter africanus subsp. capensis'. International Journal of Systematic and Evolutionary Microbiology 50, 2119–25.

da Graça JV, 1991. Citrus greening disease. Annual Review of Phytopathology 29, 109–36.

Grafton-Cardwell EE, Stelinski LL, Stansly PA, 2013. Biology and management of Asian Citrus Psyllid, vector of the Huanglongbing pathogens. Annual Review of Entomology 58, 413–32.

Halbert SE, Manjunath KL, 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Florida Entomologist 87, 330–53.

Hu H, Davis MJ, Brlansky RH, 2013. Quantification of live 'Candidatus Liberibacter asiaticus' populations using real-time PCR and propidium monoazide. Plant Disease 97, 1158–67.

Hu H, Roy A, Brlansky RH, 2014. Live population dynamics of 'Candidatus Liberibacter asiaticus', the bacterial agent associated with citrus huanglongbing, in citrus and non-citrus hosts. Plant Disease 98, 876–84.

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Huang AL, 1987. Electron Microscopical Studies on the Morphology and Population Dynamic of Fastidious Bacteria Causing Citrus Likubin. Taipei, Taiwan: National Taiwan University, PhD thesis.
Hung SC, 2006. Ecology and Vectorship of the Citrus Psyllid in Relation to the Prevalence of Citrus Huanglongbing. Taipei, Taiwan: National Taiwan University, PhD thesis.
Hung TH, Wu ML, Su HJ, 1999. Development of a rapid method for the diagnosis of citrus greening disease using the polymerase chain reaction. Journal of Phytopathology 147, 599–604.
Hung TH, Wu ML, Su HJ, 2000. Identification of alternative hosts of the fastidious bacteria causing citrus greening. Journal of Phytopathology 148, 321–6.
Hung TH, Hung SC, Chen CN, 2004. Detection by PCR of Candidatus Liberibacter asiaticus, the bacterium causing citrus Huanglongbing in vector psyllids: application to the study of vector–pathogen relationships. Plant Pathology 53, 96–102.
Inoue H, Ohnishi J, Ito T et al., 2009. Enhanced proliferation and efficient transmission of Candidatus Liberibacter asiaticus by adult Diaphorina citri after acquisition feeding in the nymphal stage. Annals of Applied Biology 155, 29–36.
Kogenaru S, Yan Q, Riera N et al., 2014. Repertoire of novel sequence signatures for the detection of Candidatus Liberibacter asiaticus by quantitative real-time PCR. BMC Microbiology 14, 39.
Li WB, Hartung JS, Levy L, 2006. Quantitative real-time PCR for detection and identification of Candidatus Liberibacter species associated with citrus huanglongbing. Journal of Microbiological Methods 66, 104–15.
Li WB, Levy L, Hartung JS, 2009. Quantitative distribution of 'Candidatus Liberibacter asiaticus' in citrus plants with citrus huanglongbing. Phytopathology 99, 139–44.
Manjunath KL, Halbert SE, Ramadugu C, 2008. Detection of 'Candidatus Liberibacter asiaticus' in Diaphorina citri and its importance in the management of citrus huanglongbing in Florida. Phytopathology 98, 387–96.
McClean APD, Schwarz RE, 1970. Greening or blotchy-mottle disease of citrus. Phytophylactica 2, 177–94.
Miyakawa T, 1980. Experimentally-induced symptoms and host range of citrus likubin (greening disease) in Taiwan, mycoplasma-like organisms, transmitted by Diaphorina citri. Annals of the Phytopathological Society of Japan 46, 224–30.
Morgan JK, Zhou L, Li WB, 2012. Improved real-time PCR detection of 'Candidatus Liberibacter asiaticus' from citrus and psyllid hosts by targeting the intragenic tandem-repeats of its prophage genes. Molecular and Cellular Probes 26, 90–8.
Pelz-Stelinski KS, Brlansky RH, Ebert TA, 2010. Transmission parameters for Candidatus Liberibacter asiaticus by Asian Citrus Psyllid (Hemiptera: Psyllidae). Journal of Economic Entomology 103, 1531–41.
Tatineni S, Sagaram US, Gowda S et al., 2008. In planta distribution of 'Candidatus Liberibacter asiaticus' as revealed by polymerase chain reaction (PCR) and real-time PCR. Phytopathology 98, 592–9.
Tsai CH, Hung TH, Su HJ, 2008. Strain identification and distribution of citrus Huanglongbing bacteria in Taiwan. Botanical Studies 49, 49–56.
Ukuda-Hosokawa R, Sadoyama Y, Kishaba M, 2015. Infection density dynamics of the citrus greening bacterium 'Candidatus Liberibacter asiaticus' in field populations of the psyllid Diaphorina citri and its relevance to the efficiency of pathogen transmission to citrus plants. Applied and Environmental Microbiology 81, 3728–36.
Wang Z, Yin Y, Hu H, 2006. Development and application of molecular-based diagnosis for 'Candidatus Liberibacter asiaticus', the causal pathogen of citrus huanglongbing. Plant Pathology 55, 630–8.

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A Note from the Senior Editor

Matt Dickinson

We would like to take this opportunity to acknowledge the hard work of all our Editorial Board members, technical readers and production team, and also the efforts of all the anonymous reviewers that we have used during the past year. We thank them all for their invaluable contributions, and for helping to maintain the standards and continued success of the Journal. In 2017, Richard Cooper, Monica Höfte and Tobin Peever stepped down from our Editorial Board and we particularly thank them for their work over many years.

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