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ORIGINAL ARTICLE

Effects of *Candidatus Liberibacter asiaticus* on the fitness of the vector *Diaphorina citri*

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Abstract

Aims: The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama transmits the bacterium '*Candidatus Liberibacter asiaticus*' (Las), which causes citrus huanglongbing (HLB) disease. Although many studies have been conducted on the biology of ACP on different host plants, few have taken the plant, Las bacteria and the vector insect within one context to evaluate the effects of Las on the fitness of ACP under field conditions. Understanding the relationship between Las and ACP is critical for both ACP and HLB disease management.

Methods and Results: We estimated the development and survival of ACP immatures, the longevity and fecundity of ACP female adults in four treatments (Las-positive or -negative ACP on Las-infected and -free citrus plants). Las-positive ACP immatures developed significantly faster on Las-infected citrus than those on Las-free plants. The fecundity and longevity of Las-positive female adults were also greater, or longer on Las-infected citrus shoots, whereas the survival of Las-positive immatures was significantly lower on Las-infected citrus shoots, compared to those that developed on Las-free plants. Similarly, the intrinsic rate of population increase (r_m) was highest (0·1404) when Las-positive ACP fed on Las-infected citrus shoots and the lowest (0·1328) when the Las-negative ACP fed on Las-free citrus shoots.

Conclusions: Both the Las infection in ACP and citrus plants had obvious effects on the biology of ACP. When compared to the Las infection in ACP insects, the Las infection in citrus shoots had a more significant effect on the fitness of ACP.

Significance and Impact of the Study: To efficiently prevent the occurrence and spread of HLB disease, it is critical to understand the ecological basis of vector outbreaks and disease incidence, especially under field conditions. Thus, this study has increased our understanding of the epidemiology of HLB transmitted by psyllids in nature.

Introduction

Huanglongbing (HLB) or citrus greening disease, caused by a phloem-limited bacterium '*Candidatus Liberibacter asiaticus*' (Las), is the most destructive disease of citrus worldwide, (Halbert and Manjunath 2004; Bové 2006). HLB disease was first discovered in the Chaoshan area of Guangdong province, China (Lin 1956). The typical symptoms of HLB-infected citrus include chlorotic leaves, twig dieback, fruit drop, misshapen small fruits, and lower internal fruit quality and eventual tree death (Bové 2006; Gottwald *et al.* 2007). It is estimated that HLB has now occurred in more than 40 countries resulting in hundreds of millions of citrus plants becoming infected, and often leading to death (Bové 2006; Grafton-Cardwell *et al.* 2013). On average, the citrus yield can decline by 30–100% depending on the severity of HLB (Iftikhar *et al.* 2016). Citrus trees start to manifest the symptoms of HLB after they are infected with the bacterium 6–12 months (young plant) or 2–3 years (old trees) later. To date, there is no efficient method to cure the disease (Grafton-Cardwell *et al.* 2013). Therefore, HLB has caused huge economic losses in citrus production and has drawn extensive attention of plant protection scientists.

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), a phloem feeding insect pest, is an already known insect vector of Huanglongbing bacterium Las in Asia and North America (Halbert and Manjunath 2004; Yang *et al.* 2006; Gottwald 2010). In China, ACP was first described in Taiwan in 1907, but its distribution has continuously increased over the last two decades due to the international long-distance seedling trade of citrus (Grafton-Cardwell *et al.* 2013). It has been reported in most parts of south China, including Guangdong, Guangxi, Taiwan, Fujian, Zhejiang, Jiangxi, Hunan, Guizhou, Yunnan and Sichuan provinces (Yang *et al.* 2006). In the United States, ACP has become established in Florida since its introduction in 1998 (Halbert and Manjunath 2004) and in Texas since 2001 (French *et al.* 2001). Nowadays, HLB is of great concern to the citrus industry in China, USA and Brazil due to the wide distribution of the vector insect and the high amount of HLB bacterium present in citrus orchards (Li *et al.* 2009).

Over the past two decades, increased studies have reported on the molecular detection of Las pathogen (Tatineni *et al.* 2008; Ammar *et al.* 2011a,b), the acquisition and persistence of Las in the psyllid vector (Huang *et al.* 2004; Pelz-Stelinski *et al.* 2010; Luo *et al.* 2015), the host plant range and preference of ACP (Nurhadi 1988; Nehru *et al.* 2004; Yang *et al.* 2006; Nava *et al.* 2007; Tsagkarakis and Rogers 2010; Westbrook *et al.* 2011), the life cycle and reproduction of ACP (Tsai and Liu 2000; Fung and Chen 2006; Nava *et al.* 2007; Hall *et al.* 2011; Alves *et al.* 2014), the flight activity and dispersal (Sakamaki 2005; Arakawa and Mivamolo 2007; Boina *et al.* 2009; Hall and Hentz 2011) as well as the interaction between Las, endosymbionts and the vector insect (Fagen *et al.* 2012; Nakabachi *et al.* 2013; Hoffmann *et al.* 2014; Lashkari *et al.* 2014). These studies promoted the understanding of the dynamics of Las and the biology of ACP; however, there is considerable variation in the biology of ACP reported within the literature, including its life cycle, reproduction, fitness and temperature limits. Furthermore, many of these studies related to uninfected citrus trials carried out in the laboratory, but rare studies have combined and investigated the interaction on citrus of all three components (plant-Las-ACP) within the whole context (Pelz-Stelinski and Killiny 2016).

To better define the role of Las on the fitness of ACP, in this study, we compared the biology of Las-positive and Las-negative ACP on Las-infected and -uninfected citrus, respectively, under field conditions. The biological traits of ACP under citrus field conditions not only provide information on the best timing for controlling the psyllid pest but also may lead to improved management strategies (Yang *et al.* 2010). Furthermore, HLB disease was first discovered in Guangdong province of China (Lin 1956); there maybe imperceptible evolution for Las, ACP as well as the citrus trees, so further insight into the interaction of citrus plant-Las-ACP insect vector is expected to be a valuable comparison and reference for ACP management work in other countries.

Materials and methods

Citrus psyllid

The Las-negative colony of ACP was originally collected from disease-free, *Murraya exotica* L. (Rutaceae) in the campus of South China Agricultural University (23°09′N, 113°20′E), Guangzhou, Guangdong, China in May 2014. The insects were reared for several generations on young plants of healthy *Citrus flamea* Hort. ex Tseng shiyueju, with a common name of ‘shatangju’.

The Las-positive colony of ACP was set up by releasing ACP adults on Las-infected citrus shoots (shatangju species) in a citrus grove in Zhaoqing city, Guangdong province, China (23°11′N, 112°50′E). The infection rate of HLB was 84% (42/50) in the psyllid population sampled from the infected citrus shatangju grove detected by nested-qPCR according to the description of Coy *et al.* (2014). Four or five instar nymphs of ACP were collected with a hand aspirator and reared on infected citrus shatangju. Both the Las-positive and Las-negative ACP were reared for at least 10 generations on HLB-free and -infected citrus trees, respectively, before the experiments.

Host plant

Both the HLB-free and -infected *C. flamea* shatangju trees were selected from two different experimental citrus groves in Zhaoqing as outlined previously—one grove with healthy plants and another with HLB-infected plants. The two groves were 5 km in distance from each other. The trees were all 3-year old and 1·5–2·0 m in height, separated by nylon net (60 mesh inch^{-2}) cages into two different experimental blocks. Again nested-qPCR detection was performed periodically to detect the presence or absence of Las in the shatangju citrus using the method described by Coy *et al.* (2014).

All the experiments were carried out from 15th June to 7th September 2014. The average temperature ranged between 28 and 32°C with relative humidity being approx. 75–85%. Before initiating the experiments, the citrus plants were watered and fertilized, and then pruned to encourage new shoot growth upon which the psyllids would find suitable to oviposit.

Development and survivorship of immature ACP

There were four different treatments consisting of Las-positive and Las-negative ACP (named as P+ and P−), Las-infected and -free citrus trees (named as C+ and C−) in this experiment. Forty pairs of Las-positive ACP adults (11 days old) were released into 40 nylon mesh bags (20 cm × 29 cm, 60 mesh inch^{-2}, one pair of ACP per bag) on the branches of Las-free or -infected citrus shoots respectively. For the other two treatments, again 40 pairs of Las-negative ACP adults (11 days old) were released into 40 nylon mesh bags covered on the Las-free or -infected citrus shoots. All the ACP adults were removed after a 24-h oviposition period. Eggs were counted using a hand magnifier, in which 30 eggs were separated out and marked by a nontoxic marker pen on each shoot. The developmental time of ACP eggs and nymphs was observed daily using a hand magnifier. The nymph instars were determined according to the size of the insect body and the development of wing pads (Tsai and Liu 2000). However, in this study, the 1st and 2nd instar nymphs were combined in the data analysis because their individual sizes were too small to distinguish in the field.

The developmental time of eggs of ACP under field conditions was examined and counted daily, from which survivorship was calculated. Similarly, all but ten 1st instar nymphs within 6 h of egg hatch were removed on each shoot. Again, the developmental time and survivorship of 1st–2nd, 3rd, 4th and 5th instar nymphs were examined and calculated using a hand magnifier. Missing nymphs were excluded from the statistical analysis. All experiments were performed in five replicates and were repeated four times independently (i.e. a total of 20 replicates for each experiment).

Longevity and reproductive capacity of ACP adults

Five pairs of newly emerged adults within 6 h from each treatment above (P+C+, P+C−, P−C+ and P−C−) were collected. From these, one pair was singly aspirated into a nylon mesh bag covering the original shoots. The paired adults were removed to new shoots every 3 days. These shoots with ACP eggs deposited were removed and the eggs counted under a binocular microscope in the laboratory. Investigations continued until all psyllid adults died. Each trial was repeated four times independently (i.e. a total of 20 replicates).

Statistical analysis

Biological differences in developmental time, survivorship of immatures, adult longevity and fecundity were analysed by one-way analyses of variance (ANOVA) using the Statistical Product and Service Solutions (SPSS Inc., ver. 17.0, Chicago, IL). Before analysis, the homogeneity of the variances was checked using Levene's test. If the variances were not homogeneous, they were subjected to log or arcsine transformations to remove heteroscedasticity. Means were separated using Fisher's protected LSD tests at a significance level of $\alpha = 0{\cdot}05$. Life-table parameters of ACP including the net reproductive rate (R_0), the mean time for each generation (T), the intrinsic rate of population increase (r_m) and the finite rate of increase (λ) were calculated according to Birch (1948) using a statistical jackknife technique (Maia Ade *et al.* 2000): $R_0 = \sum l_x m_x$, $T = \sum l_x m_x x / R_0$, $r_m = l_n R_0 / T$, $\lambda = \exp(r_m)$.

Results

Development of ACP immatures

ACP eggs developed significantly faster on Las-infected citrus shoots than those on Las-free shoots, and the same trend was recorded in relation to the Las-negative ACP eggs (Table 1). For ACP nymphs, the developmental time of Las-positive ACP nymphs from 1st to 5th instar on Las-infected citrus shoots (P+C+ treatment) was the shortest (11·30 days), followed by the Las-negative ACP nymphs on Las-infected citrus shoots (P−C+ treatment) and then Las-positive ACP nymphs on Las-free citrus shoots (P+C− treatment). The Las-negative ACP nymphs on Las-free shoots (P−C− treatment) developed the slowest (12·12 days, Table 1). There was almost a 2 days difference (15·86 *vs* 17·50) between the development periods of ACP immatures from egg to adult, which indicated that Las infection had a significant effect on the development of ACP immatures (Table 1).

Survivorship of ACP immatures

The survivorship of ACP eggs in the four treatments ranged from 68·0 to 79·4%. The results showed that the survivorship of ACP eggs on Las-infected citrus shoots was much lower than that on Las-free shoots (Table 2). In the nymphal stage, although the survivorships differed greatly between different instars, within individual certain

Table 1 The developmental periods of Asian citrus psyllid immatures in different treatments (days, Mean ± SE)

Treatment	Eggs	1st–2nd instar	3rd instar	4th instar	5th instar	Total nymphs	From egg to adult
P+C+	4·56 ± 0·01c	2·87 ± 0·04a	2·26 ± 0·02c	2·62 ± 0·03a	3·55 ± 0·03c	11·30 ± 0·04c	15·86 ± 0·04d
P−C+	4·60 ± 0·02c	2·74 ± 0·03b	2·39 ± 0·05b	2·65 ± 0·04a	3·69 ± 0·05b	11·48 ± 0·06b	16·08 ± 0·06c
P+C−	4·94 ± 0·03b	2·95 ± 0·04a	2·41 ± 0·02b	2·70 ± 0·05a	3·55 ± 0·05bc	11·62 ± 0·07b	16·62 ± 0·04b
P−C−	5·34 ± 0·01a	2·87 ± 0·02a	2·68 ± 0·04a	2·66 ± 0·05a	3·92 ± 0·04a	12·12 ± 0·05a	17·50 ± 0·06a
$F_{3,76}$	378·23	6·27	27·73	0·579	16·03	40	215·86
P	<0·0001	0·001	<0·0001	0·946	<0·0001	<0·0001	<0·0001

Means within a column followed by different letters are significantly different to each by LSD test ($P \leq 0{\cdot}05$). 'P+' and 'P−' represent Las-positive and Las-negative psyllid respectively and 'C+' and 'C−' represent Las-infected and -free citrus respectively. Experiments were performed in five replicates and repeated four times independently (n = 20).

instars, there were no significant differences among the four treatments, except between nymphs in 'P+C+' and 'P−C−' treatments. In summary, for the whole developmental period (egg to adult), the survivorship of ACP on the Las-infected citrus shoots was significantly lower than that on Las-free shoots (Table 2).

Fecundity and longevity of ACP adults

Las also affected the fecundity of ACP significantly (Fig. 1, F = 14·22, P < 0·0001). The mean fecundity of Las-positive ACP developed from Las-infected citrus shoots was highest, 520·7 eggs/female, followed by the Las-negative ACP that developed from the Las-infected citrus, which was followed by the Las-positive ACP developing from Las-free trees. The mean fecundity of Las-negative ACP females developed from Las-free citrus shoots was the lowest (424·1 eggs/female) (Fig. 1).

For the periodical reproduction of ACP in the different treatments, the fecundity peak appeared on day 13, 19, 13 and 10 in the 'P+C+', 'P−C+', 'P+C−'and 'P−C−' treatments respectively. The largest 3-day fecundity per female was 57, 53, 50 and 46·9 eggs in these four treatments respectively (Fig. 2). The sex ratios (♀/♂) of ACP adults developed from the different treatments were about 1; thus, there were no significant differences in the sex ratio between the treatments.

The mean longevities of ACP females in the four treatments were similar, ranging from 43·65 to 46·50 days (Fig. 3). There was no significant difference among the

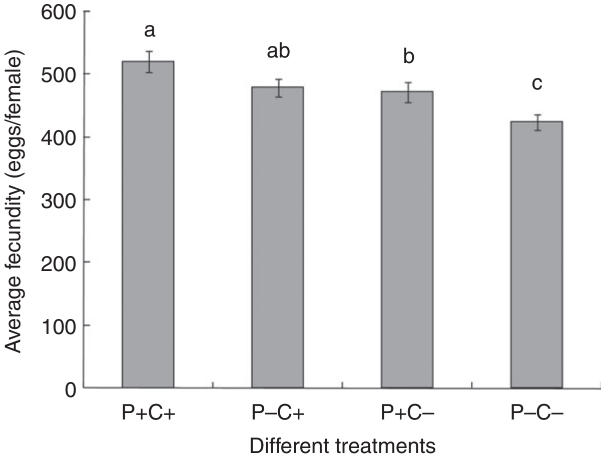

Figure 1 The fecundity of Asian citrus psyllid female adults in different treatments (eggs/female adults, Mean ± SE). The different letters over the bars are significantly different to each other by LSD test ($P \leq 0{\cdot}05$). 'P+' and 'P−' represent Las-positive and Las-negative psyllid, and 'C+' and 'C−' represent Las-infected and -free citrus respectively. Experiments were performed in five replicates and repeated four times independently (n = 20).

Table 2 The survivorship of Asian citrus psyllid immatures in different treatments (%, Mean ± SE)

Treatment	Eggs	1st–2nd instar	3rd instar	4th instar	5th instar	Total nymphs	From egg to adult
P+C+	68·00 ± 2·76b	77·20 ± 2·30a	80·27 ± 1·87a	80·99 ± 2·33a	86·78 ± 2·94b	43·60 ± 2·49b	28·96 ± 1·65b
P−C+	70·68 ± 1·90b	75·60 ± 2·52a	82·46 ± 2·11a	80·51 ± 1·76a	89·91 ± 0·97ab	45·60 ± 2·71ab	31·77 ± 1·63b
P+C−	73·32 ± 2·69ab	82·40 ± 2·60a	83·71 ± 2·13a	82·78 ± 1·29a	91·40 ± 1·97ab	53·60 ± 3·55a	40·78 ± 3·72a
P−C−	79·34 ± 2·58a	79·60 ± 1·73a	85·55 ± 1·78a	84·01 ± 1·81a	93·51 ± 1·12a	54·40 ± 3·09a	42·75 ± 2·71a
$F_{3,76}$	3·76	1·64	1·26	0·778	2·17	3·39	6·81
P	0·0140	0·1860	0·2950	0·5100	0·0980	0·0220	<0·0001

Means within a column followed by different letters are significantly different to each by LSD test ($P \leq 0{\cdot}05$). 'P+' and 'P−' represent Las-positive and Las-negative psyllid, respectively, and 'C+' and 'C−' represent Las-infected and -free citrus respectively. Experiments were performed in five replicates and repeated four times independently (n = 20).

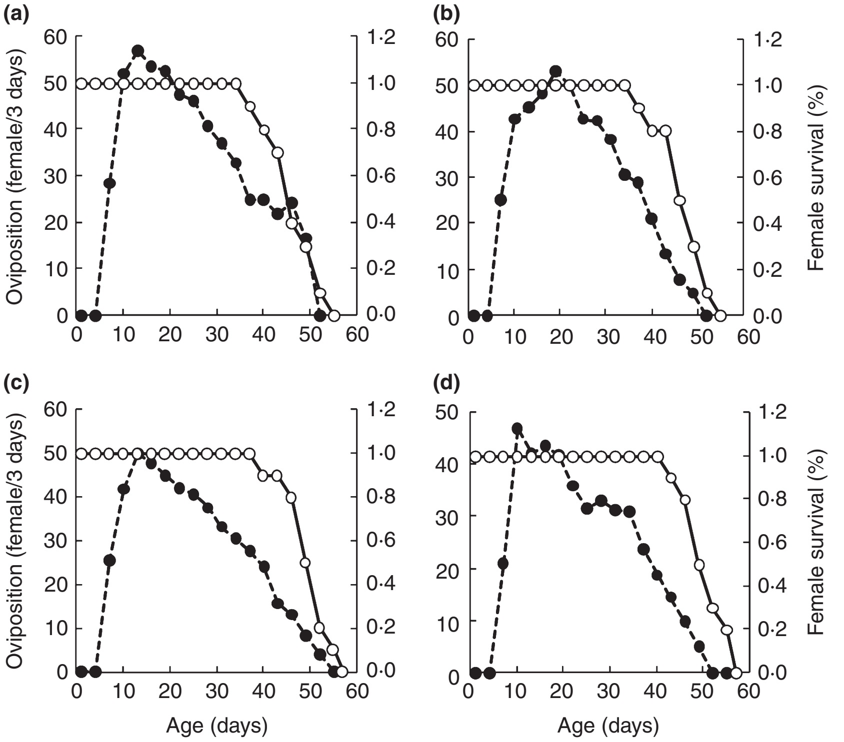

Figure 2 The oviposition (filled dot) and age-specific survival (circle) of Asian citrus psyllid female adults in different treatments. (a) P+C+ treatment, (b) P−C+ treatment, (c) P+C− treatment, (d) P−C− treatment (each experiment had 20 replicates).

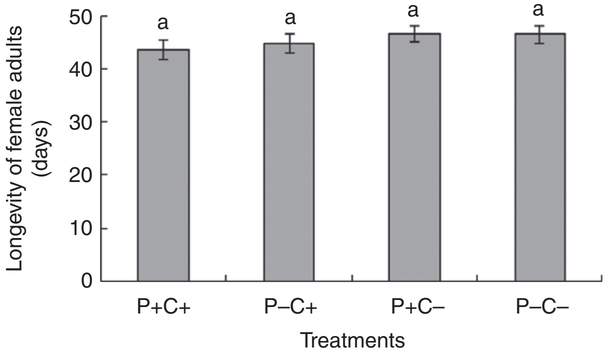

Figure 3 The longevity of Asian citrus psyllid female adults in different treatments (days, Mean ± SE). each experiment had 20 replicates.

treatments (Fig. 3, $F_{3,76} = 1{\cdot}58$ $P = 0{\cdot}201$). In addition, (as also shown in Fig. 2) the mortality of ACP female adults occurred on day 37, 37, 40 and 43 in the treatments of ‘P+C+’, ‘P−C+’, ‘P+C−’, ‘P−C−’ respectively. In general, Las infection in ACP or citrus had no apparent effect on the longevity of ACP adults.

Life-table analysis of ACP

The net reproductive rate (R_0) of the Las-positive ACP on Las-infected citrus shoots (‘P+C+’ treatment) was significantly higher than those ACP in the other three treatments (Table 3). The Las-negative ACP on Las-free citrus shoots showed the lowest reproductive rate. The mean generation time (T) of ACP in the four different treatments was very close to each other. However, for the intrinsic rate of population increase (r_{m}), similar to the trend of net reproductive rate, the highest r_{m} was recorded in the Las-positive ACP population on Las-infected citrus shoots (P+C+, 0·1404), and the lowest r_{m} was noted in the Las-negative ACP population on Las-free citrus shoots (P−C−, 0·1328). Results revealed that the infection of Las both in the citrus shoots and ACP benefits the population increase of this vector insect.

Discussion

Asian citrus psyllid and HLB disease have attracted much attention over the past two decades. Much work has investigated many aspects of the biology and control of this economically important pest (Hall *et al.* 2010; Cen *et al.* 2012; Tansey *et al.* 2015). In the current study, we focused on the life cycle of ACP on Shatangju citrus species in the Las infection context under field conditions. During the experiments, several species of parasitoids and predators were also recorded in the experimental grove (Y.T. Zhou and B.L. Qiu, unpublished data). The 60 mesh inch^{-2} nylon bag used was capable of keeping

Table 3 The life-table parameters of Asian citrus psyllid in different treatments

Treatments	R_0	T	r_m	λ
P+C+	269·7226	39·8752	0·1404	1·1507
P−C+	241·1885	39·8434	0·1377	1·1476
P+C−	240·2840	40·5786	0·1351	1·1446
P−C−	210·3536	40·2620	0·1328	1·1421

'P+' and 'P−' represent Las-positive and Las-negative psyllid, respectively, and 'C+' and 'C−' represent Las-infected and -free citrus respectively.

natural enemies away from the ACP, but it did not change or influence field the climatic conditions; meaning our findings reflect what really happens in a citrus grove independently from the impact of natural enemies.

The life cycle of ACP immatures is the key issue that has been investigated in detail in previous studies. However, the parameters are difficult to compare between these studies because of differences in citrus plant species, the temperature and the experimental locations. For example, the mean developmental periods of ACP immatures ranged from 14·06 to 28·79 days between 20 and 30°C in the study of Liu and Tsai (2000). Nakata (2006) reported that, when ACP developed from egg to adult, they required 13·4–29·5 days within the temperature range of 20–32°C. Nava *et al.* (2007) found that the duration of the egg and nymphal stages varied from 2·6 to 7·7 and from 9·4 to 35·8 days, respectively, at temperatures from 18 to 32°C. In our study, the developmental periods of ACP from egg to adult emergence ranged from 15·86 to 17·50 days, with the daily temperature fluctuating between 25 and 35°C from June to September in the Zhaoqing area. Furthermore, our results showed that Las-positive ACP developed significantly faster than negative individuals, further confirming the findings of Pelz-Stelinski and Killiny (2016). Liu and Tsai (2000) reported that the survivorship of ACP between 25 and 30°C was 73·7–83·9%, and that survivorship at 28°C was the highest. Nava *et al.* (2007) recorded the survivorship of ACP between 25 and 30°C as 66·8–69·4%, but it was sharply reduced to 5·7% when the temperature increased to 32°C. Alves *et al.* (2014) found that the survival of ACP was largely affected by host plants, varying from 32·64 to 65·90% on six different host plants at 25 ± 2°C. Compared to these three laboratory experiments, the field investigation of Paiva and Parra (2012) showed that the egg to adult survival varied from 1·7 to 21·4%. In our field experiments, the egg to adult survival of ACP ranged from 28·96 to 42·75%; much higher than the field investigation of Paiva and Parra (2012) but distinctly lower than those in the laboratory experiments. The differences in survival might be due to the Las infection status and population genetic diversity of ACP, as well as the different climatic conditions including the environmental humidity. Unfortunately, it is not possible to conclude the contribution of Las infection on the survival of ACP, as there was no Las infection information in the three previous studies.

As for the longevity and fecundity of ACP adults, Nava *et al.* (2007) reported a range of 30·96–32·42 days for ACP females and 166·22–348·37 eggs/female on three different citrus host plants at 24 ± 2°C. The investigation of Liu and Tsai (2000) revealed that the mean longevity of ACP females at 25–33°C were 28·7–39·7 days; longevity decreased as the temperature increased. The mean fecundity of ACP in their study was 316–748 eggs/female at 25–30°C; but only 67 eggs/female at 33°C. In the study of Alves *et al.* (2014), the longevity of ACP females ranged from 28·3 to 34·3 days, and the mean fecundity was 231·8 to 380·7 eggs/female; varying between each of the two populations on different host plants. In contrast, this study recorded the mean fecundity of ACP females to vary from 424·10 to 520·70 eggs/female, which is within the range of Tsai and Liu (2000) but higher than the results of Nava *et al.* (2007) and Alves *et al.* (2014). The mean longevities of ACP in the current study were 43·65–46·65 days, which were evidently longer than those in the previous studies. In summary, for the life cycle of ACP, the Las-positive ACP exhibited more fecundity and longer longevity, but shorter developmental time and lower survivorship. All this suggests that ACP displays a trade-off mode in its life-history when responding to Las infection.

The intrinsic rate of population increase (r_m) of ACP varies in different studies, for example, it was 0·0697–0·1232 on three different host plants in the study by Nava *et al.* (2007); 0·1145–0·1380 on five different host plants in the study by Alves *et al.* (2014) at 22–27°C; and 0·130–0·199 at 25–30°C recorded by Liu and Tsai (2000). Our study recorded the field r_m of ACP varied from 0·1328 to 0·1404 being within the ranges that have been previously reported. Furthermore, our findings reveal that, comparing the infection of Las in ACP insects, the infection of Las in citrus shoots had more significant effects on the fitness of ACP, due to the higher r_m of ACP on Las-infected shoots than on the Las-free shoots. To suggest a mechanism for the differences, we presume that infection of Las changed the nutrition of the citrus shoots which in turn affected the biology of ACP.

Razi *et al.* (2011) found that among the 116 citrus trees from 43 orchards located in Punjab, Pakistan, nitrogen and zinc were significantly higher ($P \leq 0{\cdot}05$) in HLB-infected trees than those HLB-free citrus trees. Zhao *et al.* (2013) revealed that significantly higher populations of eggs and adults were found in Las-infected shoots than

Las-free shoots of citrus, suggesting that the odours of Las-infected shoots are more attractive to citrus psyllid than uninfected shoots. Wu *et al.* (2015) detected the movement of ACP adults between Las-infected and healthy citrus plants. Their results suggest that, ACP adults when first confronted with a choice are more attracted to infected citrus because of the colour, but subsequently they move to healthy citrus perhaps because of either the poor nutrition or a feeding barrier in the infected hosts. This behaviour appears to facilitate the pathogen's spread. Compared with healthy shoots, (+)-epi-bicyclosesquiphellandrene was the only component detected in Las-infected shoots; while the level of sucrose, fructose and glucose were significantly higher in Las-infected shoots than in healthy shoots.

In this study, developmental period and survivorship of the ACP immatures and reproductive output of ACP adults differed significantly in the four treatments. This might be due to the infection of citrus with Las disrupting the nutrient balance of the host plants. It is suggested that the Las bacteria prefer the fructose in phloem sap and this changed the host plant sugar metabolism; the low level of fructose resulted in the steady accumulation of glucose (Andre *et al.* 2005; Fan 2010). Thus, glucose may play a key role in the development of the psyllid.

Las infection can also change the nutrition and enzyme activities of ACP, which may also influent the fitness of the psyllid insect. For example, Tiwari *et al.* (2011) found that the activities that quantify glutathione transferase and general oxidase in Las-infected ACP were lower than those in uninfected ACP, by comparing the cDNA libraries of Las-positive and -negative ACP nymph and adults. Vyas *et al.* (2015) reported that the Las can mediate the alteration of adult psyllid nutrition and metabolism as well as the development and immunity of nymphs.

In summary, our current work has revealed the effects of Las on the fitness of ACP by experiments under field conditions, which have further confirmed the findings of Cen *et al.* (2012) and Pelz-Stelinski and Killiny (2016). ACP has a shorter generation time and lays more eggs on Las-infected citrus shoots. The infection of citrus by Las is more advantageous to ACP than the infection of Las in this vector insect itself. To efficiently prevent and manage HLB disease, it is critical to understand the ecological basis of vector outbreaks and disease incidence, especially under field conditions. Thus, our current study has increased our understanding of the epidemiology of HLB transmitted by psyllids in nature. To disrupt the advantages of either Las-positive ACP or Las-infected host plants maybe the next pathway to control ACP and thus mitigate HLB in the field. Furthermore, a recent study found that not all Las-infected psyllids had the ability to transmit Las; only after the quantity of Las in the psyllid attained the threshold (~10^6), can the vector transmit the Las to citrus plants successfully (Ukuda-Hosokawa *et al.* 2015). Equally, the efficiency of Las acquisition by ACP adults was shown to be the highest on mature leaves than on young leaves and new shoots (Luo *et al.* 2015). Similarly, the phloem sap feeding mealybug (*Ferrisia virgata*) can carry Las, but it cannot cause disease in periwinkle or citrus hosts (Pitino *et al.* 2014). Therefore, further studies concerning the increase in the Las transmission threshold of ACP transmits to citrus plants, and a better understanding to reveal the mechanism of host plant immunity from these non-ill feeding of Las-infected mealybug (*F. virgata*) will also be innovative tactics needed for new HLB management to prevent HLB from spreading.

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Conflict of Interest

No conflict of interest declared.

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