An Evaluation of Plant Genotypes for Rearing Asian Citrus Psyllid (Hemiptera: Liviidae)

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Source: Florida Entomologist, 99(3) : 471-480

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.099.0320

An evaluation of plant genotypes for rearing Asian citrus psyllid (Hemiptera: Liviidae)

David G. Hall and Matthew G. Hentz*

Abstract

The Asian citrus psyllid vectors bacteria responsible for a serious citrus disease known as huanglongbing (also known as citrus greening). Many research endeavors on huanglongbing are dependent on a steady supply of Asian citrus psyllid, which can be facilitated using laboratory or greenhouse colonies maintained on an Asian citrus psyllid host plant. The choice of a plant species may be influenced by the flushing characteristics of a genotype, particularly if the goal is to produce large numbers of psyllids. This is because Asian citrus psyllid is dependent on flush (new young leaves) for reproduction. To expedite rearing, plants can be trimmed to stimulate flush growth. We studied the flushing characteristics of 9 plant genotypes known to be highly susceptible to colonization by Asian citrus psyllid: *Afraegle paniculata* (Schumacher) Engl., *Bergera koenigii* L., *Citrus aurantiifolia* (Christm.) Swingle, *C. macrophylla* Wester, *C. maxima* (Burm.) Merr., *C. medica* L., *C. reticulata* Blanco, *C. taiwanica* Tanaka & Shimada, and *Murraya paniculata* (L.) Jack. (all: Rutaceae). When plants were trimmed at 7 mo after planting, the following produced the greatest number of flush shoots: *B. koenigii*, *C. aurantiifolia*, *C. macrophylla*, and *M. paniculata*. Pruning plants once or twice before a final trimming at 7 mo after planting did not increase the number of flush shoots produced per plant for any of the genotypes. The number of psyllids produced per flush shoot was assessed on 5 genotypes that produced good quantities of flush: *B. koenigii*, *C. aurantiifolia*, *C. macrophylla*, *C. taiwanica*, and *M. paniculata*. Although some significant differences among the genotypes were observed with respect to when new adults first began to emerge and when peak emergence occurred, the differences were relatively small (a day or two). During a winter experiment, numbers of psyllids produced per flush shoot were relatively small, but significantly greater numbers of new adults were produced on *C. aurantiifolia* than on *C. taiwanica* or *M. paniculata*. Greater numbers of adults per shoot were produced during warmer weather, with no significant differences among plant genotypes. Regardless of plant genotype or time of year and for reasons that were not clear, small percentages of adults developed wing deformities. There were no differences in sex ratio, and few differences in abdominal color, among Asian citrus psyllid reared on the tested plant genotypes.

Key Words: huanglongbing; citrus greening; insect rearing; *Bergera*; *Murraya*

Resumen

El psílido asiático de los cítricos (PAC) es un vector de bacterias en los cítricos que causa la grave enfermedad conocida como Huanglongbing (también conocido como enverdecimiento de los cítricos). Muchos de los esfuerzos de investigación sobre Huanglongbing dependen de una provisión constante de PAC, que puede ser facilitada mediante colonias de laboratorio o invernadero mantenidas en una planta hospedero del PAC. La elección de una especie de planta puede ser influenciada por las características de su interaccion con la planta durante el tiempo de «flushing» (formación de botones o brotes) de un genotipo, particularmente si el objetivo es producir un gran número de PAC. Esto es debido a que el PAC depende de las nuevas hojas jóvenes para la reproducción. Para acelerar la crianza, las plantas pueden ser recortadas para estimular el crecimiento. Se estudiaron las características de flushing de 9 genotipos de plantas que se sabe que son muy susceptibles a la colonización por el PAC: *Afraegle paniculata* (Schumacher) Engl, *Bergera koenigii* L., *Citrus aurantiifolia* (Christm.) Swingle, *C. macrophylla* Wester, *C. maxima* (Burm fil.) Osbeck, *C. medica* L., *C. reticulata* Blanco, *C. taiwanica* Tanaka y Shimada, y *Murraya paniculata* (L.) Jacq. (Todo: Rutaceae). Cuando las plantas se cortaron a 7 meses después de la plantación, los siguientes produjeron el mayor número de brotes: *B. koenigii*, *C. aurantiifolia*, *C. macrophylla*, y *M. paniculata.* La poda de las plantas una o dos veces antes de que un recorte final a 7 meses después de la siembra no aumentó el número de brotes por planta al ras producidas por cualquiera de los genotipos. Se evaluó el número de PAC producidos por brote en 5 genotipos que producen buena cantidad de brotes: *B. koenigii, C. aurantiifolia*, *C. macrophylla, C. taiwanica*, y *M. paniculata*. Aunque se observaron algunas diferencias significativas entre los genotipos con respecto a cuando los nuevos adultos empezaron a emergir y cuando se produjo el pico de la emergencia, las diferencias fueron relativamente pequeños (uno o dos días). Durante un experimento de invierno, el número de PAC producidos por brote fue relativamente pequeño, pero significativamente mayor número de nuevos adultos fueron producidos en *C. aurantiifolia* que en *C. taiwanica* o *M. paniculata*. Un mayor número de adultos por brote se produjeron durante el clima más cálido, sin diferencias significativas entre los genotipos de plantas. Independientemente del genotipo de la planta o la época del año por razones que no están claras, pequeños porcentajes de PAC desarrollaron deformidades en las alas. No hubo diferencias en la proporción de sexos, y pocas diferencias en el color abdominal, entre los PAC criados sobre los genotipos de plantas probadas.

Palabras Clave: Huanglongbing; enverdecimiento de los cítricos; cría de insectos; *Bergera*; *Murraya*

Asiatic huanglongbing is one of the most serious diseases of citrus worldwide (Bové 2006). Also known as citrus greening or yellow shoot disease, Asiatic huanglongbing is putatively caused by a bacterium '*Candidatus* Liberibacter asiaticus' transmitted by the Asian citrus psyllid, *Di-*

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Supplementary material in Florida Entomologist 99(3) (Sep 2016) is online at http://purl.fcla.edu/fcla/entomologist/browse

aphorina citri Kuwayama (Hemiptera: Liviidae) (Gottwald 2010). Huanglongbing can be a devastating citrus disease especially in sweet oranges and grapefruit, rendering trees so unhealthy that they retain little or no economic value. There is no known cure for huanglongbing. Asian citrus psyllid has spread from Asia to many areas around the world and was first found in the United States (Florida) in 1998 (Halbert & Manjunath 2004). Huanglongbing was discovered in Florida in 2005, is now endemic across this state's citrus growing regions, and has put the Florida citrus industry in serious jeopardy (Hodges & Spreen 2012; Hall et al. 2013).

Laboratory or greenhouse colonies of Asian citrus psyllid can be established for research purposes. Asian citrus psyllid is not difficult to rear in most respects, and basic information on rearing procedures has been published (Skelley & Hoy 2004). Skelley & Hoy (2004) reported rearing Asian citrus psyllid on orange jasmine, *Murraya paniculata* (L.) Jack. Asian citrus psyllid has also been reared on *Citrus aurantium* L. (Mann et al. 2011); *Citrus limon* Burm. (Hall et al. 2016); *Citrus macrophylla* Wester (Hall & Richardson 2013); *Citrus medica* L. (Hall et al. 2016); *Citrus sinensis* L. (Mann et al. 2011; Liu et al. 2015); and *Bergera koenigii* L. (Simmons et al. 2013) (all: Rutaceae). There may be many other candidate genotypes for rearing Asian citrus psyllid. For example, a field study of 87 genotypes within the Rutaceae showed that a number of these were vastly favored over others by Asian citrus psyllid for colonization (Westbrook et al. 2011) including the 9 genotypes listed in Table 1. A key to rearing Asian citrus psyllid is to strategically trim plants to produce new flush shoots, simply defined as new leaf growth (Hall & Albrigo 2007). This is because Asian citrus psyllid only oviposits on newly emerging flush leaves, and nymphs only develop on flush (Husain & Nath 1927). The flushing characteristics of some plant species may be better than others for rearing Asian citrus psyllid. Intuitively, the more flush shoots a plant produces, the greater the Asian citrus psyllid production potential.

The purpose of research presented here was to compare the 9 plant genotypes listed in Table 1 as candidate Asian citrus psyllid rearing hosts with 2 primary objectives. One objective was to characterize each of the 9 plant species with respect to growth, architecture, and flush production under greenhouse conditions. The second objective was to evaluate Asian citrus psyllid production rates on 5 of the host plant species under greenhouse conditions. The ultimate goal of the research was to gather and present information pertinent to establishing or refining a rearing program for Asian citrus psyllid.

Materials and Methods

GENERAL GREENHOUSE ACTIVITIES

Assessments of genotype growth, architecture, and flushing characteristics were conducted in a conventional greenhouse with evaporative cooling and gas heat systems. Asian citrus psyllid production on 5 of the 9 plant genotypes was investigated in a hoop house that had been converted into a conventional greenhouse with an evaporative cooling system but no heater. Seeds of each plant species were obtained in 2012 and again in 2013 from the United States Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository for Citrus & Dates (NCGRCD, Riverside, California) and planted during late winter each year in individual plastic cells (3.8 cm diameter by 21 cm tall) (SC-10 super cell "Cone-tainers", Stuewe and Sons, Tangent, Oregon) containing steamed potting mix (Pro-Mix BX, Premier Horticulture, Inc., Quakertown, Pennsylvania). After planting, the "Cone-tainers" were watered on an as-needed basis and fertilized weekly with a general purpose 20N-10P-20K water-soluble fertilizer mix (Peters Professional, The Scotts Company, Marysville, Ohio). The seedlings were repotted during early summer into larger pots containing steamed Pro-Mix BX—2.54 L pots were used in 2012 (model CP59R from Stuewe and Sons) and 3.8 L pots were used in 2013 (model C300S, Universal Enterprises Supply Corp., Pompano Beach, Florida). Each year for the first 7 mo of growth, the plants of each genotype were maintained in a large group with genotypes positioned one after another along a greenhouse bench. As a preventative measure against spider mites, broad mites, thrips, and other pests, the seedlings were treated on a monthly basis usually with a tank mix of insecticide soap [40 mL per gallon (3.785 L), M-Pede (Gowan Company, LLC, Yuma, Arizona)] and petroleum oil [20 mL per gallon (3.785 L), Citrus Soluble Oil (Loveland Products, Inc., Greeley, Connecticut)] but occasionally with dicofol [6 mL per gallon (3.785 L), Kelthane MF (Dow AgroSciences LLC, Indianapolis, Indiana)] or abamectin [5 mL per gallon (3.785 L), Epimek 0.15 EC (Syngenta Crop Protection, LLC, Greensboro, North Carolina)], the latter primarily for thrips control.

GENOTYPE GROWTH, ARCHITECTURE, AND FLUSH PRODUCTION

2012 Experiments. The objectives were to assess and compare growth of the 9 genotypes (Table 1) and numbers of flush shoots produced when the plants were trimmed 7 mo after planting. Seeds of each genotype were planted on 12 Mar, and large numbers of seeds germinated for 7 genotypes, but only 5 *A. paniculata* and 3 *C. aurantiifolia* seeds germinated. Two experiments ensued, one with the 7 genotypes for which large numbers of plants were available and a second for the other 2 genotypes. For the experiment with 7 genotypes, 20 plants similar in physical appearance and size were selected from each genotype on 1 Oct (7 mo after planting). In each experiment, height and number of branches per plant were assessed just before the plants were trimmed to stimulate flush. Trimming was accomplished by cutting back the main stem of each plant to a height of 32 cm above the soil surface in each pot, and any remaining branches were trimmed at least half way to the main stem. The 32 cm height was chosen because the potted plants trimmed

Table 1. Nine plant genotypes heavily colonized by Asian citrus psyllid under field conditions (from Westbrook et al. 2011).

Scientific name	Cultivar / common name	Group	CRC[a]
Afraegle paniculata (Schumach.) Engl.	—	Citrus relative	297
Bergera koenigii L.	Curry leaf	Citrus relative	3165
Citrus aurantiifolia (Christm.) Swingle	Mexican lime	Lime	1710
Citrus macrophylla Wester	Alemow	Papeda hybrid	3842
Citrus maxima (Burm.) Merr.	Mato Buntan	Pummelo	3945
Citrus medica L.	Diamante	Citron	3523
Citrus reticulata Blanco	Tein Chieh	Mandarin	2590
Citrus taiwanica Tanaka & Y. Shimada	Nansho daidai	Sour orange	2588
Murraya paniculata (L.) Jack	Orange jasmine	Citrus relative	1637

[a] Citrus Research Center, Riverside, California accession number.

to this height conveniently fit inside a BugDorm-2 cage (BD2120F, MegaView Science Education Services Co., Ltd., Taichung, Taiwan), a cage we have often used for rearing Asian citrus psyllid (Hall et al. 2007) and from here on referred to as the rearing cage.

For Experiment 1, there were 4 replications with 5 plants of each genotype following a randomized complete block (RCB) design. Experiment 2 followed the same procedures as Experiment 1, with the 8 plants randomly grouped together in the greenhouse. In each experiment after the plants were trimmed, the number of branches per plant was reassessed and then the plants were examined 9 and 16 d after trimming to count the number of flush shoots suitable for oviposition. The plants in Experiment 1 were trimmed a second time about 9 wk later on 3 Dec, and the number of flush shoots suitable for oviposition on a daily basis was counted every 2 or 3 d through to the 18th day after trimming (6 observation days total).

Data from Experiment 1 were subjected to analysis of variance for the RCB with repeated measures (PROC GLM, SAS Institute 2010), and mean comparisons were investigated using the Ryan-Einot-Gabriel-Welsch Multiple Range Test. Data from Experiment 2 were analyzed using a *t*-test (PROC TTEST, SAS Institute 2010). Correlation analyses (Pearson's coefficient) were conducted between numbers of branches and flush shoots per plant using PROC CORR (SAS Institute 2010). All statistical tests were conducted at the $P = 0.05$ level of significance.

2013 Experiments. The objectives and basic experimental procedures were the same as for the 2012 experiments but included 4 different pruning schedules prior to a final trimming at 7 mo after planting: (1) no prior pruning, (2) pruned at 3 mo after planting, (3) pruned at 5 mo after planting, or (4) pruned at both 3 and 5 mo after planting. These different pruning schedules were investigated with the hypothesis that pruning 1 or 2 times before the final trimming would promote greater numbers of branches per plant, which in turn would increase the number of flush shoots produced when the plants were trimmed at 7 mo after planting.

Seeds of each of the 9 genotypes were planted on 19 Feb (for this experiment, seeds of *M. paniculata* were obtained locally). More than 90% germination occurred among seeds of *C. aurantiifolia*, *C. macrophylla*, *C. maxima*, and *M. paniculata* (these 4 genotypes were subjected to all 4 pruning schedules in a RCB experiment with 7 replications of each pruning schedule–genotype combination); 60 to 74% germination occurred among seeds of *C. taiwanica* and *C. medica* (there were only enough plants to subject these genotypes to 3 of the pruning schedules, which was accomplished in a second RCB experiment with 3 replications of each pruning schedule-genotype combination); 24% germination occurred among seeds of *C. reticulata* (this genotype was subjected to 2 pruning schedules, with 5 replications of each pruning schedule); but no germination occurred among seeds of *A. paniculata* or *B. koenigii*.

Each time plants were pruned (28 May, 18 Jul, or both), the main stem of the plant and any branches present were pruned back 50%. Plant height and number of branches per plant were determined on 10 Sep (7 mo after planting), immediately after which the plants were subjected to the final trimming following the same procedures as in 2012. After trimming, the number of branches per plant was reassessed, and then the number of flush shoots suitable for oviposition was counted every 2 or 3 d through to the 22nd day after trimming (8 observation days total). Data from the 2 RCB experiments were subjected to analysis of variance for the RCB with repeated measures (PROC GLM), and mean comparisons were investigated using the Ryan-Einot-Gabriel-Welsch Multiple Range Test. Data from the *C. reticulata* experiment were analyzed using a *t*-test (PROC TTEST).

ASIAN CITRUS PSYLLID PRODUCTION

The primary objectives of research on Asian citrus psyllid production were to assess and compare plant genotypes with respect to (1) Asian citrus psyllid production potential per flush shoot and (2) Asian citrus psyllid development rates from oviposition to emergence of new adults. In addition, of interest were biological parameters associated with adult psyllids reared on each genotype including sex ratios, abdominal color (newly emerged adults are usually either grey-brown or blue-green; Wenninger & Hall 2008), and the occurrence of adults with wing deformities, the latter of which have sometimes been observed within Asian citrus psyllid colonies. Five plant genotypes from Table 1 were selected for the study based on the relatively large quantity of flush they produced during the 2012 and 2013 experiments: *B. koenigii*, *C. aurantiifolia*, *C. macrophylla*, *C. taiwanica*, and *M. paniculata*.

A greenhouse colony of Asian citrus psyllid was established on each plant genotype by using adults from colonies reared on *C. macrophylla*. For each of the 5 genotypes, 2 plants with flush appropriate for oviposition were placed into a rearing cage and ~500 adults were introduced and allowed to oviposit. Three to five days later, the adults were removed from the cage and immatures were allowed to develop to the adult stage. Some of these new adults were transferred to another plant of the same genotype and allowed to reproduce. There were usually 2 or 3 colonies being maintained on each genotype at any given time.

After establishing these colonies, 3 experiments were conducted to compare Asian citrus psyllid production on each plant genotype. The experiments were similar with respect to procedures but differed with respect to the time of year when they were conducted. For each experiment, 10 plants of each genotype were trimmed to stimulate flush. Two weeks later, 5 flushing plants of each genotype were selected and each was placed individually into a rearing cage. The experiment followed the RCB design with 5 replications. The number of flush shoots per plant was standardized to 3 (3 shoots were used because this was the maximum number of shoots produced on some *B. koenigii* plants in the 1st experiment).

Each plant was infested with 3 females and 2 males per flush shoot for 24 h (a clear plastic sandwich bag was placed on each branch with shoots, a vial with the adults was introduced, and the open end of the bag was stapled shut). The adults were 10 to 14 d old. After removing the adults along with the empty vial and bag, each plant and the interior of its cage were subsequently monitored until new adults first began emerging, at which point new adults were collected daily. Data collected included the number of adults emerging each day, their sex, the color of their abdomen, and the number with wing deformities.

The experiment was conducted during winter 2015 (oviposition 13–14 Jan), late spring 2015 (oviposition 28–29 Apr), and mid-summer 2015 (oviposition 28–29 Jul). However, for the mid-summer experiment, adults were left on 4 *B. koenigii* plants for an additional day because few or no eggs were present on 29 Jul.

Analyses of variance were conducted using PROC GLM, and mean comparisons among genotypes were investigated using the Ryan-Einot-Gabriel-Welsch Multiple Range Test. Percentage data were arcsine transformed for the analyses (Gomez & Gomez 1984). Correlation analyses (Pearson's coefficient) were conducted between numbers of psyllids produced per shoot and numbers/percentages of adults with wing deformities using PROC CORR.

Results

Supplementary figures for this article are available online at http://purl.fcla.edu/fcla/entomologist/browse. The figures in the supplementary document are mentioned in the text below as Suppl. Figs. 1 to 11.

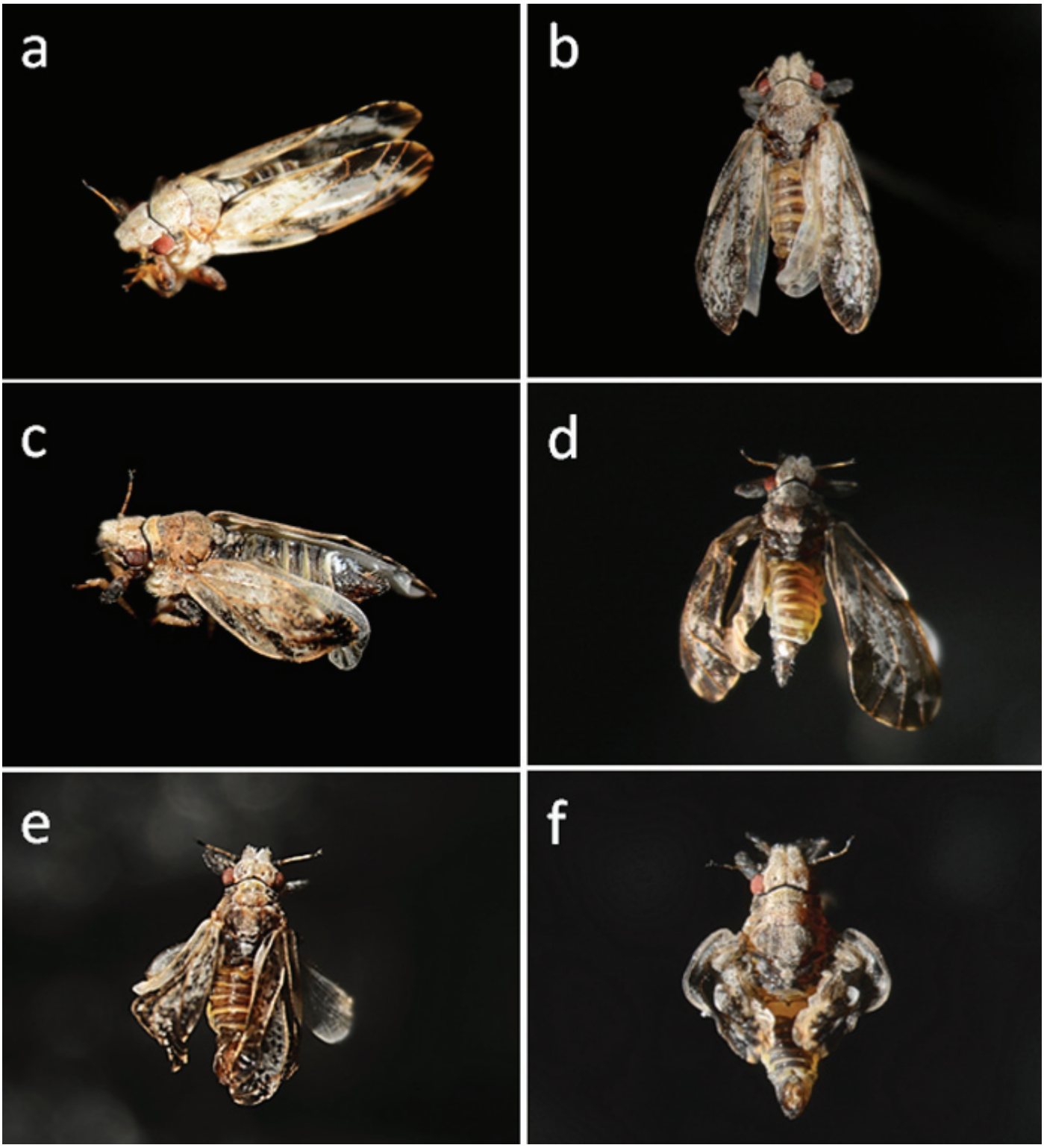

Fig. 1. Adult Asian citrus psyllids with wing deformities. (a) Normal adult. (b–d) Mild to moderate wing deformities. (e–f) Severe wing deformities.

GENOTYPE GROWTH, ARCHITECTURE, AND FLUSH PRODUCTION

2012 Experiments. Mean ± SE daily air temperature in the greenhouse during the 7 mo from planting to trimming was 30.1 ± 0.1 °C. During the 16 d after the plants were trimmed, daily air temperatures in the greenhouse averaged 30.1 ± 0.3 °C. Additional information on air temperatures is available online (Suppl. Fig. 1).

At 7 mo after planting over both experiments, *C. medica* and *C. aurantiifolia* were the tallest plants while *B. koenigii* was the shortest (Table 2; Suppl. Fig. 2). During this growth period, *C. aurantiifolia* produced the most branches followed by *C. macrophylla* and *M. paniculata*. Greater than 75% of the individual plants within 5 species had at least 1 branch: *A. paniculata*, *C. macrophylla*, *C. aurantiifolia*, *C. taiwanica*, and *M. paniculata*. Fewer than 17% of individual *B. koeni-*

Table 2. Growth, architecture, and flush production by 9 plant genotypes utilized as reproductive hosts by Asian citrus psyllid (2012). Plants were trimmed to a height of 32 cm on 1 Oct, 7 mo after planting. For Experiment 1, *n* = 20 plants per genotype. For Experiment 2, *n* = 5 *A. paniculata* plants and *n* = 3 *C. aurantiifolia* plants.

	Mean ± SE[a]					
Genotype	Plant height (cm) before the Oct trimming	Branches per plant before the Oct trimming	Branches per plant after the Oct trimming	Stem diameter (mm)	Number of flush shoots observed per plant[bc]	Number of leaves or leaflets per flush shoot or leaf 16 d after trimming
			Experiment 1			
B. koenigii	52.8 ± 3.7d	0.1 ± 0.1d	0.1 ± 0.1c	6.0 ± 0.2c	12.5 ± 0.9b	11.8 ± 0.1a
C. macrophylla	80.0 ± 4.0c	5.8 ± 0.3a	5.6 ± 0.3a	7.8 ± 0.4ab	14.0 ± 0.6b	6.7 ± 0.4b
C. maxima	76.8 ± 3.2c	0.1 ± 0.1d	0.1 ± 0.1c	8.4 ± 0.3ab	2.8 ± 0.5d	8.7 ± 0.6b
C. medica	120.5 ± 2.8a	0.7 ± 0.1d	0.4 ± 0.1c	9.2 ± 0.3a	4.4 ± 0.3d	7.2 ± 0.5b
C. reticulata	77.1 ± 1.3c	0.1 ± 0.1d	0.1 ± 0.1c	7.1 ± 0.3bc	3.9 ± 0.2d	7.6 ± 0.5b
C. taiwanica	95.8 ± 6.0b	2.2 ± 0.2c	1.7 ± 0.1b	7.9 ± 0.4ab	7.5 ± 1.0c	10.7 ± 0.5a
M. paniculata	91.0 ± 3.1bc	4.0 ± 0.6b	1.3 ± 0.1b	7.5 ± 0.3b	18.9 ± 1.2a	7.0 ± 0.2b
			Experiment 2			
A. paniculata	96.2 ± 2.0a	2.2 ± 0.5b	0.0 ± 0.0b	10.3 ± 0.3a	7.8 ± 0.5b	8.7 ± 0.5a
C. aurantiifolia	107.9 ± 8.5a	11.7 ± 2.3a	6.0 ± 0.6a	7.9 ± 0.3b	15.3 ± 2.4a	9.1 ± 0.2a

[a] Within an experiment, means in the same column followed by the same letter are not significantly different ($P > 0.05$) (Ryan-Einot-Gabriel-Welsch Multiple Range Test for Experiment 1, *t*-test for Experiment 2).
[b] Means over 2 observation days, at 9 and 16 d after trimming.
[c] Shoots suitable for oviposition

gii, *C. maxima*, *C. medica*, and *C. reticulata* plants produced branches. Trimming reduced the number of branches per plant for each genotype except *C. macrophylla*, of which most branches were below the 32 cm trimming height.

Each genotype began producing flush in less than 7 d after trimming. *Murraya paniculata* produced the greatest number of flush shoots followed by *B. koenigii* and *C. macrophylla* (Table 2). In Experiment 1, a significant correlation was found between the number of branches per plant after trimming and the number of flush shoots subsequently produced by *C. macrophylla* ($r = 0.63$, $P = 0.003$, $n = 20$), *C. medica* ($r = 0.52$, $P = 0.019$, $n = 20$), and *M. paniculata* ($r = 0.60$, $P = 0.0005$, $n = 20$); no significant correlations were found for the other 4 genotypes. In Experiment 1, significantly greater numbers of leaflets per leaf or leaves per flush shoot were observed on *B. koenigii* and *C. taiwanica* than the other 5 genotypes (Table 2).

Over the 18 d after the 2nd trimming of plants in Experiment 1, *M. paniculata* and *B. koenigii* consistently had the largest number of flush shoots (Table 3). Maximum numbers of flush shoots per plant per day were significantly highest for *M. paniculata*, *C. macrophylla*, and *B. koenigii*. Among the 6 observation dates beginning 5 d after trimming, at least some flush shoots were available for oviposition for up to 14 d after trimming each genotype and for up to 18 d after trimming *B. koenigii* and *M. paniculata* (Suppl. Fig. 3).

2013 Experiments. Mean ± SE daily air temperature in the greenhouse from planting to final trimming (19 Feb through 10 Sep) was 29.6 ± 0.1 °C; daily air temperature from when plants were trimmed on 10 Sep through the last day flush shoots were counted (2 Oct) averaged 27.7 ± 0.1 °C. Additional information on air temperatures is available online (Suppl. Fig. 4).

Among plants not subjected to any pruning prior to Sep in Experiment 1, by Sep *C. aurantiifolia* and *C. medica* plants were the tallest plants whereas *C. maxima* and *M. paniculata* plants were the shortest (Table 4). As compared with plants not pruned, pruning twice during the first 7 mo of growth resulted in significant reductions in plant height for all genotypes except *C. maxima*. Among plants not pruned during the first 7 mo of growth, *C. aurantiifolia* and *C. macrophylla* produced the greatest number of branches. Regardless of how many times plants were pruned, pruning did not promote any increase in numbers of branches by 7 mo after planting in Experiment 1 with *C. aurantiifolia*, *C. macrophylla*, *C. maxima*, or *M. paniculata*, or in Experiment 2 with *C. medica*. In Experiment 2, pruning *C. taiwanica* once at 3 mo after planting resulted in a significant increase in numbers of branches by 7 mo after planting, and pruning at both 3 and 5 mo after planting resulted in greater numbers of branches. Pruning *C. reticulata* twice during the first 7 mo of growth promoted a significant increase in numbers of branches. Trimming plants back to a height of 32 cm reduced the number of branches per plant, but *C. aurantiifolia* and *C. macrophylla* retained the greatest numbers of branches.

With respect to flush production, *C. aurantiifolia* produced the greatest number of flush shoots whereas *C. maxima* produced the fewest. None of the pruning schedules promoted an increase in numbers of flush shoots per plant—this was the case for both the average and maximum number of flush shoots observed per plant per day. Among the 8 observation dates beginning 5 d after trimming, at least some

Table 3. Flush production by 7 plant genotypes after being trimmed on 3 Dec 2012. The plants were about 9 mo old and had previously been trimmed at 7 mo after planting. Five plants per replication, 4 replications.

	Mean ± SE[a]	
Genotype	Average number of flush shoots observed per plant per day[b]	Maximum number of flush shoots observed per plant per day[c]
B. koenigii	5.9 ± 0.4ab	10.4 ± 0.7ab
C. macrophylla	5.2 ± 0.4bc	13.0 ± 1.0ab
C. maxima	2.4 ± 0.2c	4.5 ± 0.6c
C. medica	2.6 ± 0.3c	5.7 ± 0.7c
C. reticulata	2.4 ± 0.2c	5.5 ± 0.3c
C. taiwanica	4.7 ± 0.3bc	8.8 ± 0.8bc
M. paniculata	8.2 ± 0.9a	14.7 ± 1.7a

[a] Means in the same column followed by the same letter are not significantly different ($P > 0.05$), Ryan-Einot-Gabriel-Welsch Multiple Range Test.
[b] Flush shoots suitable for oviposition. There were 6 observation days after trimming.
[c] Largest daily number among 6 observation days of flush shoots suitable for oviposition.

Table 4. Growth, architecture, and flush production by 7 plant genotypes utilized as reproductive hosts by Asian citrus psyllid (2013). During the first 7 mo of growth, the plants were subjected to different pruning schedules: no pruning, pruned at 3 mo of age in May, pruned at 5 mo of age in Jul, or pruned in both May and Jul. The plants were finally trimmed to a height of 32 cm in Sep after 7 mo of growth.

	Mean ± SE [a]					
Genotype[b]	Pruning schedule before the final trimming	Plant height (cm) just before the final trimming	Branches per plant just before the final trimming	Branches per plant just after the final trimming	Average number of flush shoots per plant per day[c]	Maximum number of flush shoots per plant
Experiment 1 (*n* = 7 plants per genotype per treatment)						
C. aur.	None	100.1 ± 5.1a	10.7 ± 2.0ab	8.6 ± 1.8ab	7.4 ± 0.5a	15.3 ± 1.2a
C. aur.	May	76.7 ± 6.1bc	10.6 ± 2.8ab	7.6 ± 2.0abc	6.3 ± 0.7abc	11.7 ± 1.3abc
C. aur.	Jul	68.9 ± 4.9cde	11.4 ± 1.6a	10.0 ± 1.4a	7.3 ± 0.5a	16.0 ± 1.4a
C. aur.	May/Jul	55.3 ± 5.0cdef	10.5 ± 0.8ab	9.2 ± 0.9ab	6.7 ± 0.2ab	14.2 ± 0.5ab
C. mac.	None	94.3 ± 8.2ab	6.0 ± 1.0abcde	5.3 ± 0.8bcd	4.4 ± 0.4bcde	8.6 ± 1.1cd
C. mac.	May	74.3 ± 6.2bcd	5.7 ± 0.5bcde	4.7 ± 0.5bcd	3.9 ± 0.5cde	8.6 ± 1.0cd
C. mac.	Jul	71.0 ± 4.4bcde	8.9 ± 1.2abc	8.3 ± 1.2ab	4.9 ± 0.7abcd	11.4 ± 1.4abc
C. mac.	May/Jul	60.8 ± 2.8cdef	8.3 ± 0.9abcd	7.4 ± 0.6abc	3.9 ± 0.3cde	9.6 ± 0.7bcd
C. max.	None	64.5 ± 7.6cdef	1.1 ± 0.1e	1.1 ± 0.1d	1.8 ± 0.2e	2.3 ± 0.3e
C. max.	May	49.5 ± 4.1def	2.0 ± 0.2e	2.0 ± 0.2d	2.1 ± 0.2e	3.4 ± 0.4e
C. max.	Jul	52.7 ± 4.5cdef	2.4 ± 0.2e	2.4 ± 0.2d	1.9 ± 0.3e	3.0 ± 0.4e
C. max.	May/Jul	48.1 ± 3.8ef	2.9 ± 0.5de	2.9 ± 0.5d	2.2 ± 0.5de	3.4 ± 0.8e
M. pan.	None	73.9 ± 6.3bcde	3.9 ± 1.2cde	1.1 ± 0.1d	3.8 ± 0.8cde	5.9 ± 1.0de
M. pan.	May	62.9 ± 5.2cdef	4.2 ± 0.7cde	2.7 ± 0.2d	5.6 ± 0.7abc	10.0 ± 1.7bcd
M. pan.	Jul	50.5 ± 3.6def	4.7 ± 0.7cde	3.4 ± 0.5cd	4.9 ± 0.5abcd	8.3 ± 0.7cd
M. pan.	May/Jul	39.4 ± 6.1f	4.2 ± 0.6cde	3.8 ± 0.3cd	5.4 ± 1.4abc	9.2 ± 2.3cd
Experiment 2 (*n* = 3 plants per genotype per treatment)						
C. med.	None	102.3 ± 4.4a	1.9 ± 0.4c	1.9 ± 0.4b	3.9 ± 0.3ab	6.0 ± 0.5ab
C. med.	May	93.7 ± 3.1ab	3.4 ± 0.4c	3.1 ± 0.4b	3.0 ± 0.3b	5.0 ± 0.5b
C. med.	May/Jul	77.4 ± 3.0bc	4.4 ± 0.6bc	3.6 ± 0.4b	2.6 ± 0.2b	4.6 ± 0.4b
C. tai.	None	89.8 ± 7.0ab	2.3 ± 0.5c	2.3 ± 0.5b	5.0 ± 0.4a	6.6 ± 0.5ab
C. tai.	May	69.3 ± 7.7c	5.8 ± 0.8ab	4.2 ± 0.4b	4.6 ± 0.5a	6.5 ± 0.8ab
C. tai.	May/Jul	47.2 ± 4.9d	7.0 ± 1.2a	6.3 ± 1.1a	5.4 ± 0.4a	7.9 ± 0.8a
Experiment 3 (*n* = 5 plants per treatment)						
C. ret.	None	74.5 ± 4.7a	1.6 ± 0.2b	1.6 ± 0.2b	3.0 ± 0.4a	4.2 ± 0.4a
C. ret.	May/Jul	41.1 ± 6.1b	4.6 ± 0.5a	3.8 ± 0.5a	4.0 ± 0.8a	5.2 ± 1.3a

[a]Within an experiment, means in the same column followed by the same letter are not significantly different ($P > 0.05$); Ryan-Einot-Gabriel-Welsch Multiple Range Test for Experiments 1 and 2, *t*-test for Experiment 3.

[b]*C. aur.* = *Citrus aurantiifolia*; *C. mac.* = *Citrus macrophylla*; *C. max.* = *Citrus maxima*; *M. pan.* = *Murraya paniculata*; *C. med.* = *Citrus medica*; *C. tai.* = *Citrus taiwanica*; *C. ret.* = *Citrus reticulata*.

[c]Shoots suitable for oviposition, 8 observation days from 3 to 22 d after the final trimming.

flush shoots were available for oviposition for up to 14 d after trimming each genotype and for 18 to 22 d after trimming *C. reticulata*, *C. taiwanica*, and *M. paniculata* (Suppl. Fig. 5).

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Average and minimum daily air temperatures in the greenhouse were coolest during the winter experiment, intermediate during the spring experiment, and warmest during the summer experiment (Table 5). This trend was not the case for maximum daily air temperatures, which were as warm or warmer during the winter experiment as during the other 2 experiments. Additional information on air temperatures is available online (Suppl. Figs. 6, 7, and 8).

Winter 2015. From 25.4 to 27.8 d elapsed between oviposition and first emergence of new adults, with at least some psyllids developing moderately faster on *C. aurantiifolia* and *C. macrophylla* (Table 6). Peak emergence of new adults occurred within 27.3 to 30.0 d after oviposition, with peak adult emergence on the different genotypes generally occurring earlier on *C. aurantiifolia* and *C macrophylla* and later on *M. paniculata*. Additional information on temporal emergence of adults is available online (Suppl. Fig. 9). Significantly greater numbers of new adults per flush shoot developed on *C. aurantiifolia* than on *C. taiwanica* or *M. paniculata*.

Among new adults emerging from the different genotypes, from 49 to 58% were female with no significant differences among genotypes (Table 6). From 46 to 81% of new adults from each genotype were blue-green in color. Variability precluded declaring any significant color differences among adults from the different plant genotypes. Low percentages (≤2.1%) of new adults with wing deformities (Fig. 1) were observed among Asian citrus psyllid developing on each genotype. Over all 5 genotypes, a positive correlation was found between numbers of psyllids produced per plant and numbers of adults with wing deformities ($r = 0.76$, $P < 0.0001$, $n = 25$). Correlations between total numbers of psyllids produced per plant and percentages of adults with deformities were weakly positive across all 5 genotypes based on a significance level of $P = 0.10$ ($r = 0.33$, $n = 25$) and significantly positive for Asian citrus psyllid developing on 3 genotypes: *M. paniculata* ($r = 0.88$, $P = 0.05$, $n = 5$), *C. macrophylla* ($r = 0.98$, $P = 0.003$, $n = 5$), and *C. aurantiifolia* ($r = 0.92$, $P = 0.03$, $n = 5$).

Spring 2015. One replication of *M. paniculata* was omitted because for unknown reasons only 2 males developed on this particular plant. The average time from oviposition to emergence of new adults was 15.6 to 17.3 d, with some psyllids developing moderately faster on *B. koenigii* and *C. macrophylla* (Table 6). Peak emergence of new adults occurred within 17.1 to- 19.6 d after oviposition, with peak adult emer-

Table 5. Air temperatures during the three 2015 experiments on rearing Asian citrus psyllid on different host plant genotypes.

Time period	Daily temperature variable	Mean ± SE daily air temperature (°C) during each experiment		
		Winter	Spring	Summer
During oviposition	Mean	22.6 ± 1.3	24.8 ± 0.1	28.2 ± 0.9
	Minimum	18.0 ± 0.1	21.3 ± 0.4	25.3 ± 0.6
	Maximum	31.1 ± 0.1	28.5 ± 0.7	32.8 ± 2.7
From oviposition to first new adults	Mean	19.3 ± 0.4	23.9 ± 0.3	25.8 ± 0.3
	Minimum	11.4 ± 0.8	19.2 ± 0.6	23.3 ± 0.3
	Maximum	32.9 ± 0.5	29.3 ± 0.2	29.0 ± 0.5
From oviposition to last new adults	Mean	20.0 ± 0.4	24.9 ± 0.3	26.3 ± 0.2
	Minimum	11.2 ± 0.6	20.5 ± 0.4	23.8 ± 0.2
	Maximum	34.5 ± 0.6	29.9 ± 0.3	30.2 ± 0.5

gence per day occurring latest on *M. paniculata*. Additional information on temporal emergence of adults is available online (Suppl. Fig. 10). From 29 to 62 new adults per flush shoot were produced, with no significant differences among genotypes.

Among new adults emerging from the different plant genotypes, from 47 to 53% were female with no significant differences among genotypes (Table 6). Visual assessments of numbers of males and females emerging over time from the different genotypes indicated there were no sex differences with respect to speed of development to the adult stage, when adults first began to emerge, or when peak emergence of adults occurred (Suppl. Fig. 10). Between 86 and 96% of new adults from each genotype were blue-green in color. Significantly lower percentages of new adults from *B. koenigii* were blue-green than new adults from the other genotypes. Low percentages (≤1.2%) of new adults with wing deformities were observed among Asian citrus psyllid from each genotype. Over all 5 genotypes, a positive correlation was found between numbers of psyllids produced per plant and numbers of adults with wing deformities (r = 0.41, P = 0.04, n = 24). There was no significant correlation between total numbers of psyllids produced per plant and percentages with deformities across all 5 genotypes (r = 0.18, P = 0.39, n = 24), but a significant correlation was found for Asian citrus psyllid developing on *M. paniculata* (r = 0.96, P = 0.04, n = 4).

Summer 2015. Two replications of *M. paniculata* had to be omitted because for unknown reasons none of the immatures developed to the adult stage. Also, 1 replication of *C. taiwanica* was omitted because all 3 flush shoots aborted during the experiment. Means of from 12.6 to 14.0 d elapsed between oviposition and first emergence of new adults, with at least some psyllids developing moderately faster on *B. koenigii* than *M. paniculata* (Table 6). Peak emergence of new adults occurred within 14.7 to 15.7 d after oviposition, with no significant differences among the 5 genotypes. Additional information on temporal emergence of adults is available online (Suppl. Fig. 11). From 35 to 91 new adults per flush shoot were produced, with no significant differences among genotypes.

Among new adults emerging from the different plant genotypes, 49 to 52% were female with no significant differences among geno-

Table 6. Production parameters for a generation of Asian citrus psyllid reared on 5 host plant genotypes in a greenhouse at 3 times of the year.

Genotype	Mean ± SE [a]						
	Days to first new adult[b]	Days to peak number of new adults[b]	Days to last new adult[b]	Total psyllids produced per flush shoot	Percentage female	Percentage blue-green color morph	Percentage with deformed wings
				Winter 2015			
B. koenigii	27.0 ± 0.3ab	28.4 ± 0.5ab	36.0 ± 1.9a	18.5 ± 6.6ab	56.6 ± 4.1a	46.3 ± 6.9a	1.3 ± 0.8a
C. aurantiifolia	25.4 ± 0.4b	27.3 ± 0.2b	38.0 ± 1.0a	29.7 ± 5.2a	48.8 ± 2.1a	72.9 ± 6.7a	2.0 ± 0.7a
C. macrophylla	25.6 ± 0.5b	27.3 ± 0.3b	35.2 ± 1.1a	15.9 ± 1.9ab	52.4 ± 1.8a	80.8 ± 4.1a	1.7 ± 1.7a
C. taiwanica	27.2 ± 0.5ab	28.2 ± 0.5ab	35.6 ± 1.2a	9.1 ± 1.8b	51.2 ± 4.8a	66.5 ± 12.9a	2.1 ± 1.3a
M. paniculata	27.8 ± 0.6a	30.0 ± 1.1a	37.2 ± 2.3a	9.1 ± 2.6b	58.4 ± 7.2a	56.9 ± 8.1a	1.1 ± 1.1a
				Spring 2015			
B. koenigii	15.8 ± 0.4b	17.1 ± 0.2b	25.2 ± 1.2a	61.5 ± 10.1a	49.5 ± 0.9a	72.8 ± 2.4b	1.2 ± 0.5a
C. aurantiifolia	16.0 ± 0.3ab	17.4 ± 0.2b	24.2 ± 1.1a	58.5 ± 8.6a	51.4 ± 1.5a	96.4 ± 2.0a	1.6 ± 0.9a
C. macrophylla	15.6 ± 0.4b	17.2 ± 0.2b	23.2 ± 1.0a	60.7 ± 9.7a	53.1 ± 2.5a	90.6 ± 3.7a	1.0 ± 0.3a
C. taiwanica	16.6 ± 0.2ab	18.2 ± 0.2b	23.4 ± 0.4a	28.5 ± 4.0a	47.4 ± 2.3a	93.2 ± 1.2a	1.1 ± 0.5a
M. paniculata	17.8 ± 0.7a	19.6 ± 0.5a	24.0 ± 0.8a	29.0 ± 12.6a	50.3 ± 4.1a	93.1 ± 2.8a	0.3 ± 0.3a
				Summer 2015			
B. koenigii	12.6 ± 0.2b	15.7 ± 0.3a	23.8 ± 1.3a	91.1 ± 24.7a	51.3 ± 0.7a	57.3 ± 11.8ab	1.5 ± 0.4a
C. aurantiifolia	13.4 ± 0.2ab	14.8 ± 0.5a	26.2 ± 0.9a	65.3 ± 17.5a	51.8 ± 1.0a	90.3 ± 2.2a	2.5 ± 0.7a
C. macrophylla	13.2 ± 0.2ab	14.7 ± 0.2a	24.4 ± 1.3a	78.9 ± 4.3a	48.8 ± 1.1a	78.5 ± 7.1ab	1.7 ± 0.4a
C. taiwanica	13.5 ± 0.3ab	15.0 ± 0.0a	21.5 ± 2.1a	34.6 ± 8.7a	51.1 ± 3.2a	78.9 ± 9.5ab	0.7 ± 0.4a
M. paniculata	14.0 ± 0.0a	15.0 ± 0.0a	22.3 ± 0.3a	48.3 ± 6.5a	49.4 ± 4.1a	36.3 ± 20.9b	2.1 ± 1.0a

[a]For each time of year, means in the same column followed by the same letter are not significantly different (P > 0.05), Ryan-Einot-Gabriel-Welsch Multiple Range Test.
[b]Days from oviposition.

types (Table 6). There were no sex differences with respect to speed of development to the adult stage, when adults first began to emerge, or when peak emergence of adults occurred (Suppl. Fig. 11). From 36 to 90% of new adults from each genotype were blue-green in color. Significantly higher percentages of blue-green adults emerged on *C. aurantiifolia* than on *M. paniculata*. Low percentages (≤2.5%) of new adults with wing deformities were observed among Asian citrus psyllid from each genotype. Over all genotypes, a positive correlation was found between number of psyllids produced per plant and number of adults with wing deformities ($r = 0.80$, $P < 0.0001$, $n = 22$); there was no significant correlation between total numbers of psyllids produced per plant and percentages of adults with deformities ($r = 0.33$, $P = 0.13$, $n = 22$).

Discussion

The results of research presented here are primarily pertinent to rearing Asian citrus psyllid in the absence of '*Ca.* Liberibacter' species. This is because these bacterial pathogens may substantially alter the growth and flushing characteristics of huanglongbing-susceptible genotypes, reducing plant health to the point that it can be difficult to stimulate a diseased plant to flush. However, Asian citrus psyllids infected by the huanglongbing pathogen are required for some research projects, thus host plants that are tolerant of huanglongbing must be used for Asian citrus psyllid rearing. Genotypes that exhibit strong tolerance of the disease yet carry high titers of the pathogen would be good candidates to investigate, for example, the citrus rootstock 'US-942' (*Citrus reticulata* L. Blanco × *Poncirus trifoliata* L. Raf.) (Albrecht & Bowman 2012; Bowman et al. 2016; Hall et al. 2016). Among the genotypes in Table 1, *B. koenigii* and *M. paniculata* may not be optimal choices for rearing infected psyllids because these genotypes are generally considered relatively poor hosts of the huanglongbing bacterium (Damsteegt et al. 2010; Walter et al. 2012a,b).

GENOTYPE GROWTH, ARCHITECTURE, AND FLUSH PRODUCTION

A successful Asian citrus psyllid rearing operation is necessarily dependent on a steady supply of rearing plants, which can be influenced by a number of factors including seed germination. Large numbers of seeds of the genotypes we studied usually germinated, but there were exceptions for which reasons were unclear. Upon receiving seeds each year from NCGRCD, they were held in a refrigerator for a month or two before planting—in cases where poor germination of a genotype occurred, better germination rates might have occurred had we planted seeds sooner. Also with respect to germination, some genotypes germinated and emerged faster than others (data not presented), which is why we based plant age on planting date.

Flush production by the different genotypes was assessed 7 mo after planting by trimming seedlings to a height of 32 cm, a height chosen for a particular rearing cage and similar to the size of Asian citrus psyllid rearing plants studied by Skelley & Hoy (2004). All of the genotypes except *B. koenigii* well exceeded this height by 5 to 6 mo after planting. It was not known if the genotypes would have produced the same amount of flush if they had been trimmed earlier than 7 mo after planting. If the goal of rearing Asian citrus psyllid is to produce large numbers of psyllids, plant genotypes that produce large numbers of flush shoots after being trimmed would be favored. Among the 9 genotypes studied, these would include *B. koenigii*, *C. aurantiifolia*, *C. macrophyll*a, and *M. paniculata*.

We hypothesized that pruning a citrus seedling during early growth might increase numbers of branches and consequently the flush-quantity potential of the plant, as pruning has been shown to enhance branch production of other plants (Anonymous 2012; Williams 2005; Wright & Kelly 2008). Pruning our plants at 3, at 5, or at both 3 and 5 mo after planting prior to a final trimming after 7 mo reduced plant height and in a few cases increased the number of branches, but these pruning activities did not result in increased numbers of flush shoots per plant for any genotype. One reason was that branches promoted by pruning 3 or more months after planting ended up above the 32 cm height and thus were removed by the final trimming. Pruning earlier than 3 mo after planting might encourage lower branches. Also, rather than trimming to stimulate branching, other procedures might be more effective for producing lower branches and thus greater numbers of flush shoots, for example, countering apical dominance by bending plants over when they are 2 to 4 mo old. Another approach for increasing the number of flush shoots available in a cage of Asian citrus psyllid colony is to use 2 or 3 plants per cage. *Bergera koenigii* was an interesting genotype because new plants sometimes sprouted from the roots of a potted plant—whether these could be capitalized on for increasing Asian citrus psyllid production could be explored.

Under greenhouse conditions at average daily air temperatures of 28 to 30 °C, each genotype began producing flush within about 5 d after trimming. Therefore, within this temperature range, 5 d after trimming would be about the earliest time adults could oviposit. Skelley & Hoy (2004) presented a rearing scheme for Asian citrus psyllid in which oviposition was allowed to take place over a 2 to 4 d period, after which adults were removed. This was strategic with respect to synchronizing the age of developing nymphs for propagating Asian citrus psyllid parasitoids. Restricting oviposition to a 2 to 4 d period and tracking temperatures is also strategic from the standpoint of being able to estimate when new adults will emerge and the approximate age of new adults after emergence. To maximize Asian citrus psyllid production, this 2 to 4 d oviposition period can be timed to coincide with peak flush production.

An alternative to a 2 to 4 d oviposition period is to have a colony on a plant that is trimmed every several weeks and constantly infested by adults—adults can be removed for research purposes leaving some to continue ovipositing on new flush shoots. Although adult age cannot be tracked, an advantage of this approach is that all flush shoots produced after trimming could contribute to Asian citrus psyllid production, not just those available during a 2 to 4 d period. Using this alternative rearing scheme, genotypes such as *B. koenigii* and *C. taiwanica* that produce greater numbers of leaflets per leaf or leaves per flush shoot would be expected to produce more psyllids over time than genotypes with fewer leaflets or leaves.

ASIAN CITRUS PSYLLID PRODUCTION

Evaluations of Asian citrus psyllid production on different genotypes were restricted to 5 of the 9 genotypes listed in Table 1 due to labor and space limitations. The 5 genotypes selected generally produced the most flush, and the Asian citrus psyllid production potential of a genotype is related to the quantity of flush a plant generates (Skelley & Hoy 2004).

During the oviposition step in their Asian citrus psyllid rearing program, Skelley & Hoy (2004) routinely used an infestation rate of 3 females per flush shoot for a 2 to 4 d period. They held new adults for approximately 20 d before using them during the oviposition step. We used an infestation rate of 3 females per flush shoot, with the females 10 to 14 d old and an infestation period of 24 h. Skelley & Hoy (2004) reported variable production rates ranging from 25 to 100 nymphs per flush shoot. Our Asian citrus psyllid production rates per flush shoot were of a similar range and magnitude and also variable. Larger sample

sizes than we studied would be required for better statistical estimates of numbers of psyllids produced per flush shoot and for better comparisons among the genotypes.

Few psyllids were produced per shoot on any genotype during the winter experiment but larger numbers were produced during the spring and summer experiments. These production differences were largely attributed to air temperatures, with average temperatures coolest during the winter experiment and warmest during the spring and summer experiments. Reasons for reduced production rates during the winter would have included reduced oviposition rates. Working with Asian citrus psyllid held at constant temperatures on young seedlings of 'Duncan' grapefruit (*Citrus paradisi* Macf.), Hall et al. (2011) reported that single females oviposited an average of 15 eggs per 24 h at a temperature of 20 °C compared with 30 eggs per 24 h at 25 °C, or 40 eggs per 24 h at 30 °C. Additionally, adults 10 to 14 d old developing during cooler weather may be behind in reproductive maturity (lay fewer eggs) than adults of the same age developing at warmer temperatures. If the goal of rearing Asian citrus psyllid is to consistently produce as many as possible within a given time frame, obviously the rearing site should be kept reasonably warm during the winter, which is more difficult in a greenhouse like the one we used. Although significantly greater numbers of adults were produced on *C. aurantiifolia* than on *C. taiwanica* or *M. paniculata* in the winter experiment, data from the spring and summer experiments were too variable to declare any significant differences among these or the other 2 genotypes in numbers of psyllids produced.

Establishing a set of quality control variables for an Asian citrus psyllid rearing program would be important with respect to assessing and maintaining the general health of a colony. The sex ratio was consistently about 1:1 in each of 3 experiments with no significant differences among plant genotypes. Abdomen color is another possible quality control variable, although the biological significance of abdomen color is poorly understood. In general among 3 experiments, with few exceptions most new adults were blue-green regardless of plant genotype. Low percentages of adults with wing deformities were observed regardless of time of year—there were no significant differences among plant genotypes in percentages of adults with wing deformities. Other quality control variables that could be taken into consideration include longevity, reproductive potential, and morphology (e.g., body size).

It was unclear why some adults developed wing deformities (Fig. 1). It could have been a consequence of a nymph being crowded by others as it molted to the adult stage, and in fact during the winter 2015 experiment in some cases we found significant correlations between numbers of psyllids produced per plant and percentages of adults with deformities. There was less evidence during the other 2 experiments that deformities were related to overcrowding, and Asian citrus psyllid production rates per shoot during these experiments were larger than during the winter experiment. Wing deformities in honeybees have been associated with a virus known as Deformed Wing Virus (Iflaviridae) (de Miranda & Genersch 2010). However, bees with Deformed Wing Virus die soon after emergence, which was apparently not the case for Asian citrus psyllid with wing deformities that we observed. Wing development in some insects including Asian citrus psyllid is regulated by a number of proteins, hormones, and their analogs, notably those associated with the abnormal wing disc (*awd*) gene (El-Shesheny et al. 2013). Working on RNA interference strategies for Asian citrus psyllid control, El-Shesheny et al. (2013) showed that topical applications to 5th instar nymphs of *awd* proteins resulted in adults with malformed wings. As a follow-up to our greenhouse studies, an investigation is warranted into factors causing Asian citrus psyllid wing deformities and the incidence of adults with deformities in other settings.

Acknowledgments

We thank Anna Sara Hill, Charlene Peck, Kathy Moulton, Patrick Legler, Joe Albano (USDA-ARS, Fort Pierce, Florida) and Sandy Allan (USDA-ARS, Gainesville, Florida) for their contributions to this project. The manuscript was improved by input from 2 anonymous reviewers. Mention of a trademark or proprietary product is solely for the purpose of providing specific information and does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable. USDA is an equal opportunity provider and employer.

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