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

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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: Agraeele paniculata (Schumacher) Engl., Bergera koenigi L., Citrus aurantifolia (Christm.) Swingle, C. macrophylla Wester, C. maxima (Burm.) Merr., C. medicu 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. koenigi, C. aurantifolia, 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. koenigi, C. aurantifolia, 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. aurantifolia 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 hospedera del PAC. La elección de una especie de planta puede ser influenciada por las características de su interacción 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: Agraeele paniculata (Schumacher) Engl., Bergera koenigi L., Citrus aurantifolia (Christm.) Swingle, C. macrophylla Wester, C. maxima (Burm fil.) Osbeck, C. medicu 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. koenigi, C. aurantifolia, 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. koenigi, C. aurantifolia, 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. aurantifolia 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; cria 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-
aphorina citri Kuwayama (Hemiptera: Liviidae) (Gottwald 2010). Huang-longing 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 (Hus-sain & 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 objective 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 flushing 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”, Stewart 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 Stewart and Sons) and 3.8 L pots were used in 2013 (model C3005, 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.

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 | CRCa |
|------------------------------|------------------------|-------|------|
| Afraegele paniculata (Schumach.) Engl. | —                      | Citrus relative | 297  |
| Bergera koenigii L.          | Curry leaf              | Citrus relative | 3165 |
| Citrus aurantifolia (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 tawanonica Tanaka & Y. Shimada | Nansho daidai       | Sour orange | 2588 |
| Murraya paniculata (L.) Jack | Orange jasmine          | Citrus relative | 1637 |

*Citrus Research Center, Riverside, California accession number.
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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. aurantifolia, 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.
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. koenigii*
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. tainwanica 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. tainwanica 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 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.

| Genotype       | Plant height (cm) before the Oct | Branches per plant before the Oct | Branches per plant after the Oct | Stem diameter (mm) | Number of flush shoots observed per plant | Number of leaves or leaflets per flush shoot or leaf per plant |
|----------------|----------------------------------|----------------------------------|---------------------------------|-------------------|------------------------------------------|-------------------------------------------------------------|
| 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. tainwanica  | 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                                                  |

### 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.

| Genotype       | Average number of flush shoots observed per plant per day | Maximum number of flush shoots observed per plant per day |
|----------------|-----------------------------------------------------------|----------------------------------------------------------|
| 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. tainwanica  | 4.7 ± 0.3bc                                               | 8.8 ± 0.8bc                                               |
| M. paniculata  | 8.2 ± 0.9a                                               | 14.7 ± 1.7a                                               |

*Within an experiment, means in the same column followed by the same letter are not significantly different ($P > 0.05$).

*Means over 2 observation days, at 9 and 16 d after trimming.

*Shoots suitable for oviposition.
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).

**ASIAN CITRUS PSYLLID PRODUCTION**

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 occurring on the different genotypes generally occurring earlier on *C. aurantiifolia* and *C. macrophylla* and later on *C. 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 percentages of adults with deformities for unknown reasons only 2 males developed on this particular plant.

| Genotype | 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 | Maximum number of flush shoots per plant |
|----------|------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| *C. aur.* | None | 100.1 ± 5.1 | 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 | 3.9 ± 0.7abc | 11.4 ± 1.4abc |
| *C. mac.* | May/Jul | 60.8 ± 2.8def | 8.3 ± 0.9bcd | 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 ± 0.4d |
| *C. max.* | Jul | 52.7 ± 4.5def | 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 ± 0.8cd | 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 |

| Genotype | 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 | Maximum number of flush shoots per plant |
|----------|------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| *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 |

| Genotype | 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 | Maximum number of flush shoots per plant |
|----------|------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| *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 |

*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.
**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*.

*Shoots suitable for oviposition, 8 observation days from 3 to 22 d after the final trimming.*
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 5. Air temperatures during the three 2015 experiments on rearing Asian citrus psyllid on different host plant genotypes.

| Time period                  | Daily temperature variable | Winter          | Spring         | Summer         |
|------------------------------|----------------------------|-----------------|----------------|----------------|
|                              |                            | Mean ± SE (°C)  | Mean ± SE (°C)| Mean ± SE (°C) |
| 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     |

### 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           | Days to first new adult | Days to peak number of new adults | Days to last new adult | 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 ± 1.9b                           | 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 ± 7.1a                       | 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                  |

*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.

*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 adult from each genotype. Over all genotypes, a positive correlation was 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 ‘Co. 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 x 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 (Damsteeg 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 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. macrophylla, 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, counteracting 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
significantly greater numbers of adults were produced on
is more difficult in a greenhouse like the one we used. Although sig-
rearing site should be kept reasonably warm during the winter, which
produce as many as possible within a given time frame, obviously the
temperatures. If the goal of rearing Asian citrus psyllid is to consistently
opposing during cooler weather may be behind in reproductive maturity
- at a temperature of 20 °C compared with 30 eggs per 24 h at 25 °C,
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