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Authors: Paris, Thomson M., Rohde, Barukh B., Allan, Sandra A., Mankin, Richard W., and Stansly, Philip A.

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SYNCHRONIZED REARING OF MATED AND UNMATED *DIAPHORINA CITRI* (HEMIPTERA: LIVIIDAE) OF KNOWN AGE

Thomson M. Paris[1], Barukh B. Rohde[2], Sandra A. Allan[3], Richard W. Mankin[3*] and Philip A. Stansly[4]
[1]Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620, USA

[2]Biology Department, Hunter College of the City University of New York, 695 Park Avenue, New York, NY 10065, USA

[3]United States Department of Agriculture, Agriculture Research Service, Center for Medical, Agricultural, and Veterinary Entomology, 1700 SW 23rd Dr, Gainesville, FL 32608, USA

[4]Entomology and Nematology Department, University of Florida/IFAS, Southwest Florida Research and Education Center, 2685 SR 29N, Immokalee, FL 34142, USA

*Corresponding author; E-mail: richard.mankin@ars.usda.gov

The Asian citrus psyllid (ACP) (*Diaphorina citri* Kuwayama; Hemiptera: Liviidae) is a major pest of citrus, vectoring an apparently devastating bacterial pathogen, '*Candidatus* Liberibacter asiaticus' (Halbert & Manjunath 2004; Hall & Gottwald 2011). Although ACP nymphs can be reared on young terminal shoots and adults on mature leaves of citrus plants (Ammar & Hall 2011), the lack of artificial diets hinders capability to rear large numbers of ACP for behavioral investigations to improve the effectiveness of management programs (Hall et al. 2010). In addition, rearing procedures that produce ACP of known age and mating status are needed for a wide range of behavioral studies, including mating and orientation. Typically, mating of ACP begins on day 3 after emergence (Wenninger & Hall 2007) and mated males are less likely than unmated males to employ vibrational communication in searching for females (Wenninger et al. 2009). Age and mating status also may affect ACP dispersal (Aubert & Hua 1990) and light-attraction behaviors (Sétamou et al. 2012). To obtain sufficient ACP of known age and/or mating status for such studies, we modified procedures developed by Wenninger & Hall (2007) for individually rearing ACP nymphs on isolated plants.

Isolation chambers to rear individual nymphs or small groups of adults (Fig. 1) were constructed from 21-cm-long, 3.75-cm diam cone-tainers (Model SC10, Steuwe & Sons, Inc. Tangent, Oregon). A cap was prepared for each cone-tainer by cutting a 10-cm-long section from 3.75-cm diam polyethylene terephthalate glycol tubing (1½" Ultra Thin Wall X 4.000 Tube PETG-000, Part Number: PRT.012-1.644, ClearTec Packaging, MOCAP Corporation, Park Hills, Missouri) and covering it with an inner-fit plug made of polypropylene (1½" Poly Plug Natural, Part Number: PRP1.500NAT, ClearTec Packaging, MOCAP Corporation, Park Hills, Missouri). A hole (3.50-3.60 cm diam) was drilled in the cover plug and covered in either of 2 sizes of insect-proof mesh (7.87 holes per cm and 13 holes per cm, length and width), fastened with hot glue. Four 1-cm-diam holes were drilled 0.75 to 1.25 cm from the top of the tube, and also covered in mesh to enable maintenance of moderate levels of relative humidity (RH). High humidity has been observed previously to reduce ACP survivability (Aubert 1990).

Initially, each cone-tainer was filled with potting soil (reed sedge peat, horticultural perlite, ground dolomitic limestone, and slow release fertilizer 0.10-0.08-0.06, Stan Green All Purpose Potting Mix, Jungle Growth, LLC.) in which a single citrus seedling was planted. Soil moisture was maintained by placing cone-tainers in racks over trays containing sufficient water to cover drainage. Once the seedling reached a height of 4-5 cm, the cap was placed in position and the chamber was ready for use for rearing ACP. When the seedling outgrew the cap, it was trimmed and used again. Isolation chambers were kept under light intensities between 3,000-8,000 lux.

Psyllids were obtained from the USDA-ARS, Gainesville colony of ACP of mixed age (left column, Fig. 2) confined inside screened cages (BugDorm, MegaView Science Co., Taichung, Taiwan). The ACP were allowed to oviposit for a minimum of 14 days on 3-5, 0.5-m-tall *Murraya paniculata* (L.) Jack (Sapindales: Rutaceae) plants. Plant foliage had been clipped 14 days previously to induce new flush. The plants were maintained on a 16:8 h L:D cycle at a light intensity varying between 11,000-13,000 lux, watered 3 days per week, and fertilized with 20:20:20 fertilizer once per week. A HOBO U14 Data Logger (Model U14-002, Onset Computer Corporation, Pocasset, Massachusetts) was used to monitor the mean and standard error of temperature (26.7 ± 2.1 °C) and RH (34 ± 2.5%) of the greenhouse and 3 isolation chambers (25.9 ± 0.1 °C; 74 ± 0.7% RH). As in previous ACP rearing procedures of Skelley & Hoy (2004), ants were controlled when necessary

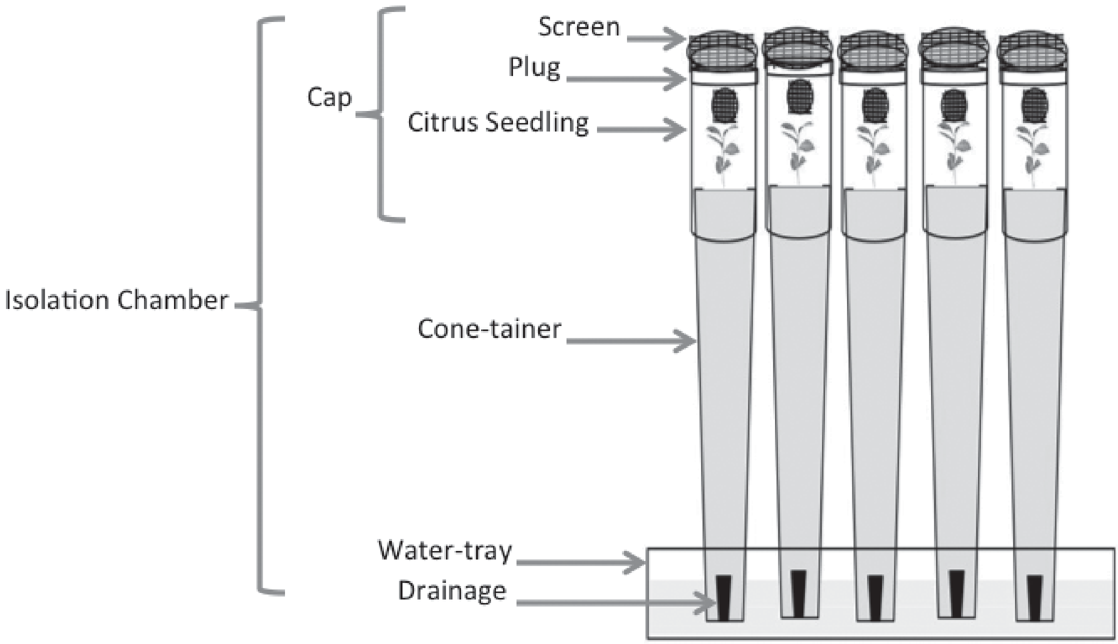

Fig. 1. Isolation chambers used for rearing unmated and mated Asian citrus psyllids of known age.

by standing the screen-cage shelf legs in containers filled with soapy water, and populations of other pests were reduced by aspiration or by spraying with petroleum oil and/or insecticidal soap (Safer Soap, Woodstream, Lititz, Pennsylvania).

After the 14-day oviposition period, the host plants were removed from each colony cage and rinsed with a gentle stream of water. The rinse removed most adult psyllids while retaining the nymphs and eggs on the host plant. The few adults that remained on the plant were removed by an aspirator. Now harboring only nymphs and eggs, the host plant was placed back into a cage that did not contain adults.

The final stages of the rearing process depended on whether unmated or mated ACP were needed for testing. For mated ACP, each rearing cage was checked daily for newly emerged adults (thick-dashed box, Fig. 2). New adults were removed and placed as a group onto a seedling in an isolation chamber labeled with the emergence date. The host plant was rinsed with water and then checked visually with care for adults a second time before it was placed back into the cage. In a representative sample of collections from a cage on 3 consecutive days, a mean of 37.5 adults were collected each day on the first inspection and none on the second inspection. The daily process was repeated as needed until the experiment ended. This procedure for obtaining known-age adults was similar to that described by Coombe (1981), who reared up to 3,000 white flies, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) of known age in days for studies of white fly attraction to light.

For bioassays in which unmated psyllids were needed, a single 3rd-5th stage nymph was placed into each isolation chamber. The chamber was checked daily for adult emergence and labeled with the date of emergence (thick-lined box, Fig. 2). The adult was sexed using a light microscope and then returned to the chamber until used in a bioassay. Rearing of unmated and mated ACP was synchronized by daily checking of adults and plants in cages (dashed-line boxes, Fig. 2) and isolation chambers (solid boxes, Fig. 2), and monthly cycling of cages.

The effect of insect-proof mesh size on Asian Citrus Psyllid survival was analyzed using a pairwise t-test. No significant differences in mean percentage survival were found between the 2 insect-proof mesh sizes in 6 trials at nymph counts densities of 10 [(61.67 ± 0.058% for mesh-13 vs. 56.67 ± 0.56% for mesh-7.87, df = 5, t = 1.94, P = 0.111)] and 30 [(63.89 ± 2.34% for mesh-13 vs. 63.89 ± 1.23% for mesh-7.87, df = 5, t = 1.94, P = 1)].

The new ACP rearing procedures provided benefits over previously used methods with smaller cone-tainers. First, the seedlings remained viable for a longer period and nymphs were easier to maintain in moderate levels of humidity than previously (adults placed in isolation chambers remained viable for > 30 days). The larger viewing area made it easier to find nymphs in the isolation chamber than in the smaller cone-tainers,

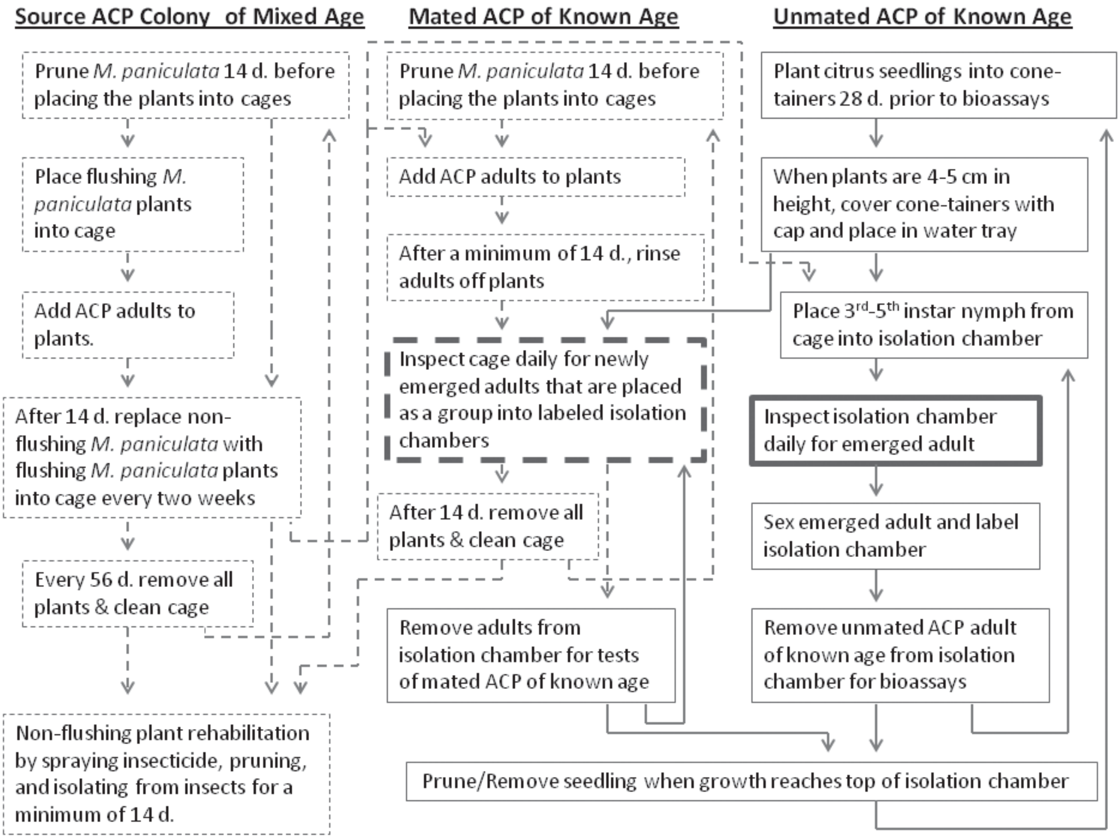

Fig. 2. Time-synchronization of activities in rearing of unmated and mated Asian citrus psyllids of known age for use in behavioral studies. Cage-associated tasks were denoted with dashed boxes and arrows, and isolation-chamber tasks denoted with solid boxes and arrows.

enabling a reduction in the time needed to check for adult eclosion. Finally, the larger isolation chambers provided space for high numbers of mated psyllids that were used in dispersal and light-attraction studies.

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Summary

Methods were developed for synchronized rearing of unmated and mated Asian citrus psyllid (ACP) (*Diaphorina citri* Kuwayama; Hemiptera: Liviidae) of known age in isolation chambers for behavioral studies. Maintenance and survival of nymphs and adults in the isolation chambers was improved over previous methods because the plants had more room to grow and the relative humidity was easier to keep within acceptable limits.

Key Words: citrus seedling, cone-tainer, mesh, *Murraya paniculata*, survivability

Resumen

Se desarrollaron métodos para la cría sincronizada del psílido asiático de los cítricos (PAC) de edades conocidas, no apareados y apareados (*Diaphorina citri* Kuwayama, Hemiptera: Liviidae) en cámaras de aislamiento para estudios de comportamiento. El mantenimiento y la sobrevivencia de las ninfas y adultos en las cámaras de aislamiento se ha mejorado con respecto a los métodos usados anteriormente, debido a que las plantas tenían más espacio para crecer y la humedad relativa fue más fácil mantener dentro de los límites aceptables.

Palabras Clave: plántulas de cítricos, recipiente, malla, *Murraya paniculata*, sobrevivencia

References Cited

AMMAR, EL-DESOUKY, AND HALL, D. G. 2011. A new method for short-term rearing of citrus psyllids (Hemiptera: Psyllidae) and for collecting their honeydew excretions. Florida Entomol. 94: 340-342.

AUBERT, B. 1990. Integrated activities for the control of huanglongbing-greening and its vector *Diaphorina citri* Kuwayama in Asia, pp. 133-144 *In* B. Aubert, S. Tontyaporn and D. Buangsuwon [eds.], Proc. 4th Intl. Asia Pacific Conf. Citrus Rehabilitation, Feb. 4-10, 1990, Chiang Mai, Thailand.

AUBERT, B., AND HUA, X. Y. 1990. Monitoring flight activity of *Diaphorina citri* on citrus and *Murraya* canopies, pp. 181-187 *In* B. Aubert, S. Tontyaporn and D. Buangsuwon [Eds.], Proc. 4th Intl. Asia Pacific Conf. Citrus Rehabilitation, Feb. 4-10, 1990, Chiang Mai, Thailand.

COOMBE, P. E. 1981. Visual Behavior of the Whitefly *Trialeurodes vaporariorum*. Ph.D. Thesis, University of Adelaide.

HALBERT, S. E., AND MANJUNATH, K. L. 2004. Asian citrus psyllid (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Florida Entomol. 87: 330-353.

HALL, D. G., SHATTERS, R. G., CARPENTER J. E., AND SHAPIRO J. P. 2010. Research toward an artificial diet for adult Asian citrus psyllid. Ann. Entomol. Soc. America 103: 611-617.

SÉTAMOU, M., SANCHEZ, A., PATT, J. M., NELSON, S. D., JIFON, J., AND LOUZADA, E. S. 2012. Diurnal patterns of flight activity and effects of light on host finding behavior of the Asian citrus psyllid. J. Insect Behav. 25: 264-276.

SKELLEY, L. H., AND HOY, M. A. 2004. A synchronous rearing method for the Asian citrus psyllid and its parasitoids in quarantine. Biol. Control 29: 14-23.

WENNINGER, E. J., AND HALL, D. G. 2007. Daily timing of mating and age at reproductive maturity in *Diaphorina citri* (Hemiptera: Psyllidae). Florida Entomol. 90: 715-722.

WENNINGER, E. J., HALL, D. G., AND MANKIN, R. W. 2009. Vibrational communication between the sexes in *Diaphorina citri* (Hemiptera: Psyllidae). Ann. Entomol. Soc. America 102: 547-555.