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Authors: Ammar, El-Desouky, and Hall, David G.

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A NEW METHOD FOR SHORT-TERM REARING OF CITRUS PSYLLIDS (HEMIPTERA: PYSLLIDAE) AND FOR COLLECTING THEIR HONEYDEW EXCRETIONS

EL-DESOUKY AMMAR AND DAVID G. HALL
Subtropical Insects Research Unit, USDA-ARS, Fort Pierce, FL. 34945

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The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is an economically important pest of citrus in the United States, Asia and other parts of the world, as a vector of huanglongbing (HLB) or citrus greening, which is considered one of the world's most serious diseases of citrus (Gottwald 2010). Additionally, large populations of ACP can damage plants directly through feeding and excretion activities; ACP feeds on citrus phloem tissues and produces copious amounts of honeydew excretions (Brlansky & Rogers 2007). ACP adults can feed on mature citrus leaves, but nymphs must have young tender flush to survive. For biological and pathogen-vector relation studies on ACP (e.g., Wenninger & Hall 2007; Pelz-Stelinski et al. 2010) rearing of single or small groups of psyllid nymphs or adults on whole citrus plants takes considerable space, time and other resources. Here, we describe a new, simpler method for short-term rearing of ACP using detached mature citrus leaves for adults and detached young terminal shoots for nymphs (Fig. 1).

ACP adults and nymphs were reared singly or in small groups (5-10 per tube) in clear plastic (polypropylene) 50-ml conical centrifuge tubes (3 cm wide and 11.5 cm long; Fisher Scientific, Pittsburg, PA). Young psyllid adults (approximately 1-wk-old) were reared on detached, mature, medium-size leaves of sweet orange (*Citrus sinensis* (L.) Osbeck var. Ridge Pineapple). Leaf sizes used for adults ranged between 3-4 cm in width and 6-8 cm in length with petioles about 2-3 cm long. The petioles of these leaves were cut diagonally with a sharp razor blade, and each inserted in a small (0.3 or 0.5 mL) microfuge tube filled with water or a piece of moistened cotton wool (Figs. 1A and 1E). A piece of Parafilm membrane was wrapped around the top of this tube and the petiole to keep insects from drowning or contact with water. The detached leaf and microfuge tube were then inserted into the rearing tube, and the psyllid adults were added to the latter. The rearing tube was covered with a screw cap that had been finely perforated by a hot needle for ventilation. For better ventilation, however, wider holes can be cut in the plastic cap and a piece of fine mesh screen placed under the screw cap to prevent escape of the psyllids. The tubes were kept in an environmental chamber at 25°C and 14 h light per day.

The clear plastic wall of the rearing tubes allowed close observation and photography of the enclosed psyllids during their various activities either by the naked eye or through a stereomicroscope with minimal disturbance (Figs. 1B-1E). For example, adults were observed feeding for long periods in their normal feeding posture (Fig. 1B) mainly on the midrib or other veins on either side of the detached leaves. They were also observed excreting honeydew droplets regularly (Fig. 1E), and occasionally laying eggs (Fig. 1B inset), although they normally prefer younger leaves for laying eggs (Brlansky & Rogers 2007). Survival of young adults under the above conditions on detached mature leaves, that were changed to fresh ones weekly, was 89, 80 and 75% after 2, 3, and 4 weeks, respectively (n = 130 adults). It was later observed, however, that mature leaves can stay fresh in the rearing tubes at least for 2 weeks.

Young ACP nymphs were reared on younger citrus leaves using the above described rearing tubes under similar conditions. We followed the survival and adult emergence of young (2nd-3rd-instar) nymphs for 1 week on the following 3 types (leaf age/size) of sweet orange leaves (Fig. 1A): (A) partially expanded young leaves (2-3 terminal leaves on a young flush shoot); (B) fully expanded tender leaves; and (C) mature mid-size leaves similar to those used for rearing adults. After gently placing a group of young nymphs on each of these three types of leaves (10 nymphs/tube), the tubes were kept horizontally for a few h to overnight to allow the young nymphs to settle and start feeding. The overall proportion of nymphs that survived for 1 week in these tubes was 77.8% in treatment A (youngest leaves), 61.1% in treatment B (young leaves), and 56.7% in treatment C (mature leaves). Chi square (χ^2) analysis indicated that survival of nymphs was significantly higher in treatment A than in treatments B or C (Table 1). The proportion of nymphs that turned into adults during 1 week of rearing was significantly higher in Treatments A and B (55.7-67.3%) compared to that in treatment C (25.5%) (Table 1). Survival and development of nymphs in treatment A are comparable to those of ACP nymphs reared on whole citrus seedlings at 25°C (Liu & Tsai 2000). Nymphs were observed

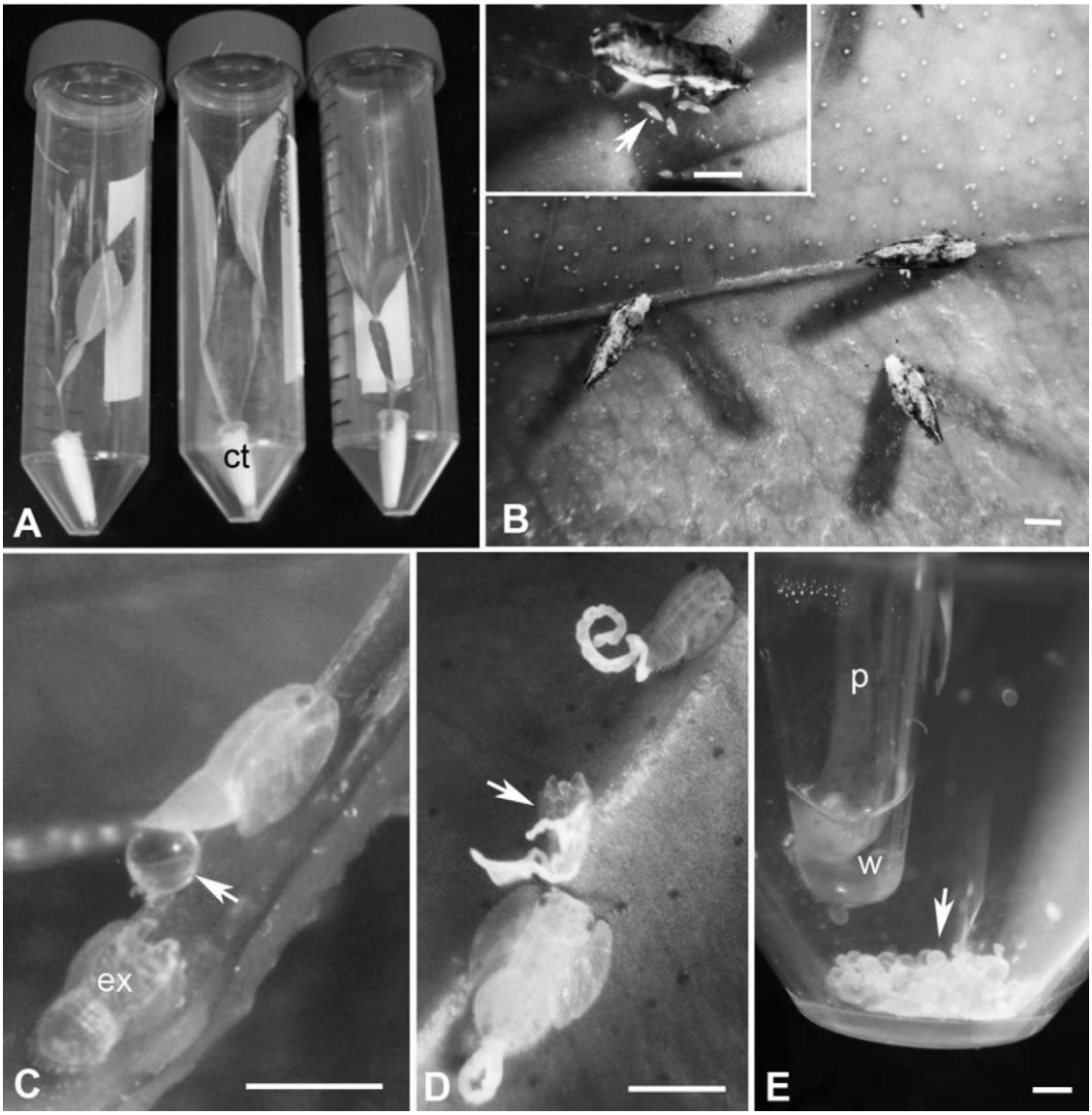

Fig. 1. A. Conical polypropylene 50-mL tubes used for rearing psyllids on citrus leaves of various ages (from left to right): a. young leaves on a flush shoot, b. fully expanded tender leaves, and c. mature mid-size leaves; mature leaves were used for rearing adults but the young leaves were more suitable for rearing young nymphs. The cut end of each leaf petiole/shoot was placed in a small microfuge tube filled with water or a piece of moistened cotton (ct). B. ACP adults in their normal feeding posture, feeding on the midrib or other veins. The inset shows an adult female and several eggs (arrow). C. Fifth instar nymph excreting a large droplet of honeydew (arrow), with an empty skin (exuvium) located behind it (ex). D. Third and fourth instars (upper and lower nymphs, respectively) excreting tubular-shaped material; arrow indicates an exuvium with tubular-shaped excretions still attached. E. Honeydew excretion droplets (arrow) accumulating in the conical bottom of the rearing tube in which 5 adults were kept for 5 d. Other abbreviations: p, petiole; w, water in the bottom of the microfuge tube. All scale bars = 1 mm.

feeding regularly for long periods, molting and excreting large amounts of honeydew in droplet or tubular forms especially on the youngest or younger leaves tested (Figs. 1C and 1D).

When the rearing tubes were kept vertical, most of the ACP honeydew excretion droplets fell down from the leaves and accumulated in the conical bottom of the rearing tubes (Fig. 1E). This can be a convenient and efficient way to collect the psyllid excretions for various studies on feeding behavior/chemistry (Hall et al. 2010). We believe that this new rearing method allows closer obser-

TABLE 1. SURVIVAL AND ADULT EMERGENCE OF YOUNG (2ND-3RD-INSTAR) NYMPHS OF *D. CITRI* FOR 1 WEEK IN REARING TUBES WITH DETACHED SWEET ORANGE LEAVES OF VARIOUS AGES.

		Trial 1		Trial 2		Trial 3		Overall**	
Attribute	Leaf Age*	No.	%	No.	%	No.	%	No.	%
Survival	A	24/30	80.0	24/30	80.0	22/30	73.3	70/90	77.8 a
	B	16/30	53.3	14/30	46.7	25/30	83.3	55/90	61.1 b
	C	27/30	90.0	11/30	36.7	19/30	63.3	51/90	56.7 bc
Adult emergence	A	18/24	75.0	17/24	70.8	4/22	18.2	39/70	55.7 a
	B	13/16	81.2	7/14	50.0	17/25	68.0	37/55	67.3 a
	C	12/27	44.4	0/11	00.0	2/19	10.6	12/51	23.5 b

*Leaf age designation: (A) Partially expanded young leaves on a young flush shoot; (B) Fully expanded young leaf; and (C) Mature mid-size leaf (Fig. 1A).

**χ^2 analysis conducted on overall proportions: For each attribute, percentages followed by different letters are significantly different ($P < 0.001$-0.003).

vation of psyllids, and can save time, space and other resources in various studies on the biology and management of ACP and probably other citrus psyllids. It can be particularly valuable for studying psyllid behavior, HLB pathogen-vector interactions, and for bioassay of biological or chemical agents against citrus psyllids.

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SUMMARY

We developed a new simple method for short-term rearing of the Asian citrus psyllid (ACP) using detached citrus leaves in 50-mL conical polypropylene tubes. Survival of young adults was 89, 80, and 75% after 2, 3, and 4 weeks, respectively, on detached mature leaves that were changed weekly. Survival and adult emergence of 2nd to 3rd-instar nymphs were significantly higher when reared on younger leaves compared to those reared on mature leaves. Honeydew excretion droplets of ACP accumulated and may be easily collected from the conical bottom of the rearing tubes. This new method allows closer observation and photography of psyllid nymphs and adults with minimal disturbance, and it can save time, space and other resources in various studies on the biology, behavior, management and pathogen-vector interactions of ACP and probably other citrus psyllids.

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