

## Research Toward an Artificial Diet for Adult Asian Citrus Psyllid

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**ABSTRACT** Research progress is reported on an artificial diet for adult Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). The primary objective was to develop a system for screening antimicrobial peptides and other potential toxic proteins for activity against adults. The base diet was a sterilized solution of sucrose (30%) and yellow-green food coloring (0.5%) in tap water. All of the studies presented were conducted at 25°C, 75% RH, and a photoperiod of 14:10 (L:D) h. Adult psyllids were <7 d old when they were transferred to diet. Addition of the food coloring was necessary to prompt adults to feed. Among the feeding trials discussed, a mean of  $69.1 \pm 3.2\%$  adults survived for 14 d on the base sucrose diet. Survival rates of males and females were similar. Adults feeding on the sucrose diet may have ingested less food than adults feeding on citrus leaf disks based on differences in quantities of adult excrements deposited in feeding chambers. However, survival of adults feeding on leaf disks over a 2-wk period was only marginally better than survival of adults feeding on the base sucrose diet, and final rates of survival of adults fed these two food sources were not significantly different.

**KEY WORDS** citrus greening disease, huanglongbing, *Diaphorina citri*, rearing

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is an important invasive pest in the United States because it vectors a bacterium responsible for a severe disease of citrus known as huanglongbing. This disease causes tree decline, renders fruit inedible, and eventually results in tree death (Halbert and Manjunath 2004, Gottwald 2007). There is no known cure for citrus huanglongbing. Disease management recommendations include an aggressive psyllid control program (Bové 2006). *D. citri* was first found in the United States during 1998 in Florida (Tsai and Liu 2000) and subsequently invaded Alabama, Arizona, California, Georgia, Louisiana, Mississippi, South Carolina, and Texas (Bech 2009). Huanglongbing was first found in the United States during 2005 in Florida (Bové 2006) and subsequently was found in Georgia, Louisiana, and South Carolina (Bech 2009).

We recently commenced research to develop an artificial diet for adult *D. citri* for the purpose of screening toxins that might be candidates for novel psyllid control tactics or for a transgenic solution to the huanglongbing problem. Davidson et al. (1996) used an artificial diet for the similar purpose of screening potential toxins to the white fly *Bemisia argentifolii*

(Bellows & Perring). Many artificial diets have been developed for plant-chewing insect species, and a large number of these diets are available commercially. In contrast, little progress has been made in developing artificial diets for rearing plant-sucking insects. Some success, however, has been reported in developing artificial diets for rearing aphid and whitefly nymphs to the adult stage (Auclair 1965, Akey and Beck 1971, Srivastava and Auclair 1971, Turner 1971, Jancovich et al. 1997, Davidson et al. 2000). The base of these aphid and whitefly diets is a sucrose solution ranging from 15 to 35% in water, to which various amino acids, vitamins and minerals (among other ingredients) have been added.

Presented here is a progress report for research on an artificial diet and feeding system for adult *D. citri*. Preliminary research indicated that a base liquid diet consisting of sucrose (30%) and food coloring in water sandwiched between two Parafilm membranes sustained relatively large percentages of adults for 2 wk or longer. The pH of this base diet was  $7.4 \pm 0.1$  (mean  $\pm$  SE). This report presents experiments conducted to assess survival of adults on the base sucrose diet with various modifications. Feeding rates were investigated in some experiments by counting numbers of psyllid excretions or feeding sites (remnants of salivary stylet sheaths through the feeding membrane). Agar can be added to the base diet to enhance observations on psyllid salivary sheaths because, when agar is present, these sheaths can be seen to extend through the feeding membrane into the diet. An experiment was therefore conducted to assess adult survival on the base sucrose diet with and without agar.

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Survival of adults on the base sucrose diet was compared with survival of adults feeding on citrus leaf disks in similar feeding chambers, as citrus leaves are the natural food source for adults. Finally, an apparatus was investigated that automated the encapsulation of the base sucrose diet in Parafilm, and survival of adults feeding on membrane-encapsulated diet was assessed. The apparatus has been used to study membrane-encapsulated insect diets by other researchers, for example Ferkovich et al. (2000) and Ferkovich and Shapiro (2004).

### Methods and Materials

The insects for these studies were obtained from a colony described by Hall et al. (2007). In brief, the colony was established during early 2000 at the USDA-ARS U.S. Horticultural Research Laboratory, Fort Pierce, FL. Originally collected from citrus, the psyllids have since been continuously reared in cages containing orange jasmine, *Murraya paniculata* (L.) Jack. The colony is maintained by transferring adults to new *M. paniculata* plants on a fortnightly schedule by using procedures similar to those described by Skelley and Hoy (2004) with no infusion of wild types to avoid contaminating the colony with the huan-glongbing bacterium.

**Base Sucrose Diet.** The base diet used in these studies was a solution of sucrose (30%), yellow food coloring (0.4%), and green food coloring (0.1%) in tap water. Amounts of materials for 40 ml of diet were as follows: sucrose, 12.0 g; yellow food coloring, 148.8  $\mu$ l; green food coloring, 49.6  $\mu$ l; and tap water, 40.0 ml.

Larger or smaller quantities of the diet were made based on component percentages. The yellow and green dyes were from McCormick & Co., Inc. (Hunt Valley, MD). The yellow dye contained water, propylene glycol, FD&C Yellow 5, FD&C Red 40, and 0.1% propylparaben. The green dye contained water, propylene glycol, FD&C Yellow 5, FD&C Blue 1, and 0.1% propylparaben. After mixing the diet ingredients, the solution was autoclaved. All diet preparation activities were conducted in a clean air hood that was wiped down with ethanol and subsequently treated with UV light for 15 min before setting up feeding chambers.

**Feeding Chambers.** A small culture dish (suspension culture dish, 35 by 10 mm, nontreated polystyrene; 430588, Corning Life Sciences, Lowell, MA) was used as the feeding chamber. For all of the studies presented here, five young ( $\leq 7$ -d-old) adults were placed into each dish and the lid was put in place. Plastic vials with five psyllids per vial were placed into a refrigerator at 5°C for 1–2 h to chill (subdue) the adults while setting up an experiment. The vials were removed one at a time, the psyllids were placed into the feeding chamber dish in the clean air hood, and a 4.8- by 2.5-cm piece of Parafilm membrane was stretched across the dish above the psyllids. A slight indentation of the membrane was created with a finger (wearing sterile disposable gloves) at the center area of the membrane, and 0.5 ml of the liquid diet was

pipetted into this center depression. A second membrane was then stretched across the first, sandwiching the diet. For all studies presented here, after setting up feeding chambers they were placed in a growth chamber at 25°C, 75% RH, and a photoperiod of 14:10 (L:D) h. Dishes were placed 24 cm from the incubator's light source, which consisted of two 20-W fluorescent light bulbs (F20T12/CW, Philips Electronics North America Corporation, Andover, MA). At this distance from the lights, light intensity just above the feeding chambers was  $\approx 2,100$  lux (1,900 foot candles).

**Adult Survival on the Base Sucrose Diet: Different Amounts of Sucrose.** Survival of adults on the base sucrose (30%) diet with food coloring was compared with survival of adults feeding on the diet with 0, 10, 20, 40, 50, or 60% sucrose and food coloring. Ten feeding chambers of each diet were prepared, and five adults (sex not determined) were introduced into each chamber. These were set up and observed daily (except weekends) for 13 d to count numbers of live psyllids. Analysis of variance (ANOVA) was conducted to compare percent survival of adults feeding on the seven diets.

**Adult Survival and Feeding Rates on the Base Sucrose Diet With Different Amounts of Food Coloring.** Survival of adults on the base sucrose diet with food coloring was compared with survival of adults feeding on the diet with half the amount of food coloring or no food coloring. Ten feeding chambers of each diet were prepared, and five adults (sex not determined) were introduced into each chamber. These were set up and observed daily (except weekends) for 14 d to count numbers of live psyllids. Feeding rates on each of the three diets were investigated by counting the number of feeding sites (salivary sheaths) within two 7.9-mm<sup>2</sup> sites on the feeding membrane from each chamber (two locations midway between the center and edge of the membrane) by using a dissecting stereomicroscope (1.0 $\times$  objective, zoom at 10 $\times$ ). To facilitate counting, a solution of Coomassie Brilliant Blue R-250 and Coomassie Brilliant Blue G-250 electrophoresis purity (both dyes from Bio-Rad Laboratories, Hercules, CA) was applied to a feeding membrane to stain salivary sheaths. Approximately 1 ml of the dye solution was pipetted into the diet between the membranes. The top membrane and dye were removed after 10 min, and the diet-side of the remaining membrane was flushed three times with deionized water before counting feeding sites.

**Adult Survival on the Base Sucrose Diet With and Without Agar.** Survival was compared among adults fed the base sucrose diet with and without agarose (product 7060, 150 mesh [Bio-Serv, Frenchtown, NJ] at 1.4% [0.56 g for 40 ml of diet]). Ten feeding dishes (replications) of each diet were prepared for each sex (five adults of either sex per replication). These psyllids were then observed daily (except weekends) for 14 d to the count numbers of live psyllids. Paired *t*-tests were conducted to compare percent survival of psyllids on the two diets.

**Survival of Adults on Grace's Medium.** Grace's insect medium (Sigma-Aldrich, St. Louis, MO) was

added to the base sucrose diet (44.0 g/liter), and survival on this diet was compared with survival of adults on the base sucrose solution. Ten dishes (replications) of each diet were set up with males and ten were set up with females (five adults per dish). The pH of each diet was determined and adjusted to 7.0 by using a solution of NaOH (40 g/liter). Feeding chambers with adults were then set up and observed daily (except weekends) for 14 d to count numbers of live psyllids. Paired *t*-tests were conducted to compare percent survival of psyllids on the two diets.

**Survival of Adults on a Sucrose Diet Containing Yeast Extract.** Survival of adults feeding on the base sucrose solution with agar was compared with this same sucrose solution containing 1, 3, or 5% yeast extract (Bacto TM yeast extract, BD Biosciences, Sparks, MD). Five dishes (replications) of each diet were set up with males and five were set up with females (five adults per dish). Adult survival was monitored over a 7-d period. ANOVA was conducted to compare percentage of survival of adults feeding on the four diets.

**Survival and Excretion Rates of Adults Feeding on Leaf Disks Versus on the Base Sucrose Diet.** Adult *D. citri* survival on leaf disks was compared with adult survival on the base sucrose diet. A fresh citrus ('Duncan' grapefruit, *Citrus paradisi* Macf.) leaf disk (23.4 mm dia.) was embedded on agarose (7 g/500 ml water) in the culture dish described above. Leaves for the study were excised from young trees maintained in a greenhouse. One leaf disk was punched from each leaf using a 2.34-cm-dia. copper pipe with a sharpened edge similar to that of a cork borer. A leaf disk was then placed on agar in each culture dish, adults were placed onto the disk, the lid of the dish was put into place, and the dish was placed into a growth chamber at 25°C, 75% RH, and a photoperiod of 14:10 (L:D) h. Ten dishes (replications) with five males and ten with five females were prepared for each diet. These were set up and observed daily (except weekends) for 14 d to count numbers of live psyllids. Numbers of psyllid excretions in each feeding chamber were assessed over the first 3 d for psyllids feeding on leaf disks and over the first 5 d for psyllids feeding on the sucrose diet. Paired *t*-tests were conducted to compare percent survival of psyllids on the two diets.

**Adult Survival on Parafilm-Encapsulated Sucrose Solution.** The base sucrose diet was prepared and encapsulated in Parafilm by using a vacuum-driven, diet encapsulation apparatus (Analytical Research Systems, Gainesville, FL) designed for a 24-chamber tray (a modified bottomless Falcon tissue culture plate, Sigma-Aldrich). Procedural details for encapsulating diet in Parafilm were similar to those described by Carpenter and Greany (1998), although these researchers used an alternative to a vacuum to stretch the membrane. In brief, the bottomless culture plate was placed on the encapsulation apparatus, a piece of Parafilm was placed on the exposed side of the culture plate and a vacuum was pulled simultaneously through each chamber, stretching the membrane into a concave shape into each chamber and sealing the membrane side of the plate. The culture plate was

**Table 1.** Mean daily percentage of survival over a 13-d period of adult *D. citri* feeding on a liquid diet consisting of water, food coloring, and various percentages of sucrose<sup>a</sup>

Diet	Mean $\pm$ SEM daily % adult survival
0% sucrose	41.2 $\pm$ 4.2d
10% sucrose	72.8 $\pm$ 3.3c
20% sucrose	90.1 $\pm$ 1.0b
30% sucrose	94.6 $\pm$ 1.2a
40% sucrose	86.8 $\pm$ 2.2ab
50% sucrose	88.6 $\pm$ 1.4ab
60% sucrose	79.8 $\pm$ 1.9bc

<sup>a</sup> Means followed by the same letter are not significantly different ( $\alpha = 0.05$ ; Tukey's test). Analyses are on arcsine-transformed percentages; raw percentages are presented.

then removed from the vacuum apparatus, placed onto a surface with the membrane side up, diet was dispensed onto the membrane associated with each chamber (0.5 ml of diet per chamber), and a piece of freezer paper (Reynolds plastic coated freezer paper, Reynolds Kitchens, Richmond, VA) was pressed against the membrane (plastic side toward the membrane), sealing the diet against the membrane. A Plexiglas plate was placed over the sticky paper, the culture plate was turned over, insects were introduced into each chamber, and the culture plate lid was attached to confine the insects in each chamber. Rubber bands were used to keep the entire feeding container together. Two trays of encapsulated diet were prepared and a single adult (sex not identified) was placed into each chamber. The trays were placed into a growth chamber at 25°C, 75% RH, and a photoperiod of 14:10 (L:D) h. These were set up and observed daily (except weekends) for 23 d to count numbers of live psyllids.

**Statistics.** Means and SEs were calculated and are presented as mean  $\pm$  SE. Percentage data were arcsine-transformed for analyses. Paired *t*-tests were conducted using PROC TTEST, and analyses of variance were conducted using PROC GLM (mean separations by Tukey's test or the Ryan-Einot-Gabriel-Welsch multiple range test,  $\alpha = 0.05$ ) (all procedures by SAS Institute 2008).

## Results

**Adult Survival on the Base Sucrose Diet: Different Amounts of Sucrose.** Mean daily percentage of survival was greatest among adults feeding on diet containing 30, 40, or 50% sucrose, with no significant differences in survival of adults on these diets (Table 1). Percentage of survival of adults feeding on diet containing 20 or 60% sucrose was significantly lower than of adults feeding on the 30% sucrose diet. At the end of the 13-d trial, 100% mortality had occurred among adults fed the diet lacking sucrose.

**Adult Survival and Feeding Rates on the Base Sucrose Diet With Different Amounts of Food Coloring.** Means of 96  $\pm$  2.7, 80  $\pm$  5.2, and 24  $\pm$  7.8% adults were alive after 6 d when they were fed the sucrose diet with 100, 50, and 0% of the standard rate of food coloring, respectively. At the end of the 14 d study, means of

**Table 2.** Mean daily percentage of survival over a 7-d period of adult *D. citri* feeding on the base sucrose diet with or without yeast extract<sup>a</sup>

Diet	Mean $\pm$ SEM daily % adult survival	
	Females	Males
30% sucrose	90.8 $\pm$ 2.9a	87.3 $\pm$ 3.1a
30% sucrose + 1% yeast extract	67.5 $\pm$ 7.9b	63.3 $\pm$ 6.3b
30% sucrose + 3% yeast extract	68.3 $\pm$ 5.9b	64.0 $\pm$ 6.7b
30% sucrose + 5% yeast extract	62.2 $\pm$ 6.1b	64.7 $\pm$ 6.6b

<sup>a</sup> Means in the same column followed by the same letter are not significantly different ( $\alpha = 0.05$ ; Ryan-Einot-Gabriel-Welch multiple range test). Analyses are on arcsine-transformed percentages; raw percentages are presented.

84  $\pm$  9.8, 60  $\pm$  11.2, and 12  $\pm$  4.4% of the adults feeding on these diets, respectively, were still alive. Means of 11.4  $\pm$  1.5, 5.8  $\pm$  0.9, and 0.2  $\pm$  0.1 salivary sheaths per 10 mm<sup>2</sup> were observed on membranes associated with 100, 50, and 0% of the standard rate of food coloring, respectively.

**Adult Survival on the Base Sucrose Diet With and Without Agar.** Mean daily percentage of survival was 78.2  $\pm$  1.7 and 88.0  $\pm$  1.0% for adults fed on the sucrose diet with and without agar, respectively. These means were significantly different ( $t = 5.8$ ,  $df = 208$ ,  $P > |t| < 0.0001$ ). Survival of adults feeding on the base sucrose diet with agar was significantly lower for both females ( $t = 5.3$ ,  $df = 103$ ,  $P > |t| < 0.0001$ ) and males ( $t = 4.7$ ,  $df = 92$ ,  $P > |t| < 0.0001$ ). At the end of the 14-d trial, mean percentage survival was 66.8  $\pm$  6.6 and 73.7  $\pm$  4.8% for adults feeding on the sucrose diet with and without agar, respectively. There was no significant difference between these means ( $t = 1.2$ ,  $df = 18$ ,  $P > |t| = 0.2$ ).

**Survival of Adults on Grace's Medium.** Means of 27.0  $\pm$  5.9 and 69.1  $\pm$  6.7% adults were alive after 14 d when they were fed the sucrose diet with and without Grace's medium, respectively. A mean daily percentage of survival of 61.0  $\pm$  2.4% was observed for adults feeding on the sucrose diet with Grace's medium compared with 86.8  $\pm$  1.3% for adults fed the base sucrose diet. These percentages were significantly different ( $t = 8.6$ ,  $df = 219$ ,  $P > |t| < 0.0001$ ). Survival of adults feeding on Grace's medium was significantly reduced for both females ( $t = 6.2$ ,  $df = 102$ ,  $P > |t| < 0.0001$ ) and males ( $t = 9.1$ ,  $df = 109$ ,  $P > |t| < 0.0001$ ).

**Survival of Adults on a Sucrose Diet Containing Yeast Extract.** Survival of adults feeding on the sucrose diet containing yeast was significantly lower than survival of adults feeding on the base sucrose diet (for females:  $F = 3.58$ ,  $df = 3$ ,  $P > F = 0.01$ ; for males:  $F = 3.60$ ,  $df = 3$ ,  $P > F = 0.02$ ) (Table 2). A mean of 73.3  $\pm$  4.7% adults survived for 7 d when they were fed the base sucrose diet compared with 11.1  $\pm$  3.5, 5.5  $\pm$  2.8, or 3.6  $\pm$  2.4% when they were fed the sucrose diet containing 1, 3, or 5% yeast, respectively.

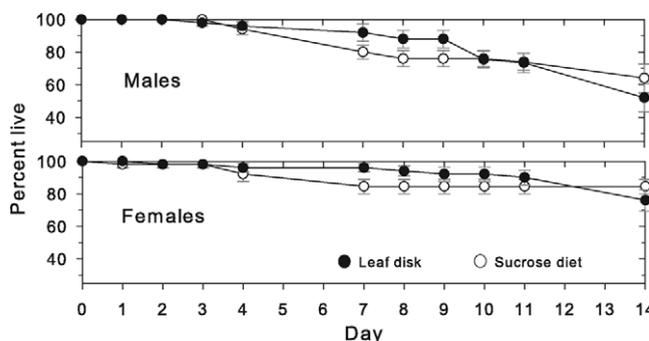
**Survival and Excretion Rates of Adults Feeding on Leaf Disks Versus on the Base Sucrose Diet.** Percentage of survival of adults was similar on the two diets for the first 4 d, but higher percentages of adults feeding on leaf disks remained alive after 7–9 d (Fig. 1). Mean daily percentage of survival was significantly lower for females feeding on the base sucrose diet than on leaf disks ( $t = -2.1$ ,  $df = 103$ ,  $P > |t| = 0.04$ ). However, there were no significant differences on any individual observation date in percentage of survival between females feeding on the sucrose diet and females feeding on leaf disks (analyses not shown). There was no significant difference in percent survival between males feeding on the sucrose diet and males feeding on leaf disks over all observation dates ( $t = -1.0$ ,  $df = 109$ ,  $P > |t| = 0.34$ ) nor on any individual observation date (analyses not shown).

Cumulative numbers of excretions associated with five females feeding on a leaf disk were relatively large after 2 d and exceeded 100 per feeding chamber by 3 d (Fig. 2). In contrast, females feeding on the sucrose diet for 5 d produced only a cumulative average of  $\approx$ 30 solid excretions. Males feeding on leaf disks for 2 d produced  $\approx$ 5 times more excretory droplets than males feeding on the sucrose diet.

**Adult Survival on Parafilm-Encapsulated Sucrose Solution.** Means of 72.9  $\pm$  2.1 and 66.7  $\pm$  0.0% adults survived on the encapsulated sucrose diet for 7 and 14 d, respectively, and 54.2  $\pm$  4.2% diet were alive after 23 d. Mean daily percentage of survival of adults over the entire 23 d trial was 71.6  $\pm$  2.4%.

## Discussion

Among the feeding trials presented here, a mean of 69.1  $\pm$  3.2% adult *D. citri* survived for 14 d on the base



**Fig. 1.** Survival of adult *D. citri* on a simple sucrose diet compared with adult survival on citrus leaf disks.

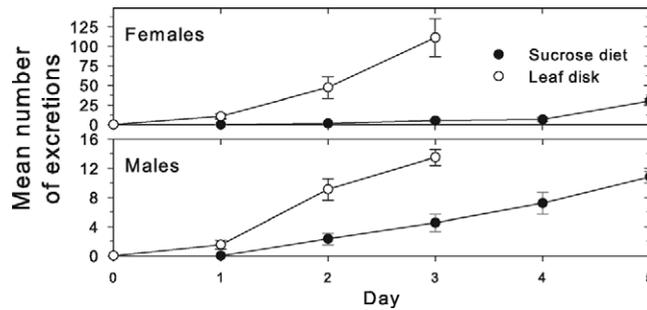


Fig. 2. Quantity of excretions associated with five adult *D. citri* feeding on a simple sucrose diet compared with five adults feeding on citrus leaf disks.

sucrose diet. Survival rates of adults feeding on the base sucrose diet were similar with respect to sex, with means of  $71.4 \pm 4.4\%$  females and  $66.8 \pm 4.6\%$  males surviving on the diet for 14 d. In feeding trials not presented in this report, low percentages of adults sometimes survived for  $\geq 40$  d on the base sucrose diet.

The particular suspension culture dish used as the feeding chamber was selected because it was small and easy to work with. However, an array of different types of vials and containers were tried in preliminary trials with little difference noted in adult survival. Some of these containers were vented and others were not.

Adult *D. citri* are strongly attracted to light, thus adults were attracted by light to the surface of the membrane containing diet. Yellow-green food coloring was an important diet ingredient apparently because it stimulated feeding. This was evident from differences in numbers of salivary sheaths observed in membranes that contained diet with different amounts of food coloring. A second advantage of adding food coloring was that excretions from psyllids turned green in color, confirming diet ingestion.

Adult *D. citri* males only produced liquid droplets whether they fed on leaf disks or the sucrose diet. These honeydew droplets were colorless when adults fed on citrus leaves. Adult females exuded a solid product similar to that exuded by nymphs (Husain and Nath 1927). These solid exudates varied in shape from spherical to squiggly strands and were white in color when females fed on leaves. Little is known about the nature of the solid excretions produced by females and nymphs or why adult males do not exude a solid product. Husain and Nath (1927) described the exudates from nymphs as a thick sugary liquid covered over with a waxy secretion from the circumanal glands, appearing as a whitish, thick translucent cord. However, the chemical constituents of the solid exudates associated with nymphs and females are largely carbohydrates and contain no lipids (R.G.S., unpublished).

In evaluating a diet for adult *D. citri*, the presence and abundance of exudates have value as indicators of feeding and consumption rates. Boina et al. (2009) used counts of honeydew droplets to make inferences on feeding activity by adult *D. citri*, although these authors made no reference to solid excretions. We

counted droplets from males and solid excretions from females in some of our experiments, but in practice this was confounded to some extent by differences in the size and shape of droplets and solid excretions. Also, adult movement in feeding chambers can disrupt and merge excretions, in general making them difficult to quantify. Weighing excretions that fall onto filter or wax paper to quantify feeding rates was tried with limited success. It might be possible to quantify excretions from adults feeding on the green diet using a spectrophotometer or a fluorimeter (for the latter, if a fluorescent dye is added to diet).

Adults feeding on the sucrose diet ingested less food than adults feeding on citrus leaf disks based on differences in quantities of adult excrements deposited in feeding chambers. However, survival of adults feeding on citrus leaf disks was only marginally better than survival of adults feeding on the base sucrose diet, and percent survival of adults fed these two food sources were statistically the same after 14 d. The average life expectancy of adult females was reported to be 40–48 d when they were maintained on plants at 25°C (Tsai and Liu 2000). Because we worked with adults that were initially no older than 7 d, we expected higher percentages of survival over time than we observed among adults feeding on either leaf disks or sucrose diet. With respect to leaf disks, lower rates of survival might be attributed to unobservable dehydration of leaf tissue (in spite of the leaf tissue being embedded on moist agar) or a breakdown of phloem constituents. Adult survival on leaves detached from trees might be reduced compared with survival on leaves not detached from trees. Reduced survival rates on both leaf disks and sucrose diet may have been associated with excessive relative humidity, as condensations were sometimes present inside feeding chambers. Jancovich et al. (1997) reported that survival of whiteflies [*Bemisia argentifolii* (Bellows & Perring)] feeding on a sucrose solution was increased by replenishing diet every 2–4 d, and survival of the pea aphid, *Acyrtosiphon pisum* (Harris), on an artificial diet was improved when diet was renewed three times a week (Akey and Beck 1971). Therefore, survival of adult *D. citri* feeding on a sucrose diet might be increased if the adults were moved into containers of fresh diet every few days.

Several artificial diet ingredients have been investigated for rearing whiteflies and aphids including sucrose, maltose, yeast, amino acids, Grace's amino acid mixture, vitamin mixtures, lipid mixtures, and minerals (Auclair 1965, Akey and Beck 1971, Turner 1971, Jancovich et al. 1997, Davidson et al. 2000). Thus we investigated some of the same ingredients for sustaining adult *D. citri*. Grace's Insect Medium contains an array of amino acids, minerals and vitamins. Compared with adults feeding on the base sucrose diet, survival of adults was reduced when they were fed a sucrose diet containing Grace's Insect Medium. Survival was also reduced when yeast extract at from 1 to 5% was added to the base sucrose diet.

The pH of the base sucrose solution averaged  $7.4 \pm 0.1$ . It remained possible that adult survival on the sucrose diet might have been improved at a different pH, although the sucrose diet pH was in the range of levels sufficient for whiteflies. Salvucci et al. (1997) reported that the optimum pH for ingestion of a 20% sucrose diet by adult whitefly *B. argentifolii* was between 6.5 and 7.5 based on a test range of 4.5–8.5. Davidson et al. (2000) reported that a dietary pH of 6.5 or 8.0 supported higher percentages of nymph development of *B. argentifolii* than a pH of 5.0.

Mold contaminants sometimes developed in the sucrose diet within several days, although the presence of mold did not seem to affect psyllid survival. Adult psyllids were the source of these contaminants, as in separate trials no mold developed on diet associated with feeding chambers in the absence of psyllids. We ran some limited trials with the sucrose diet to which methyl paraben (0.02%) or sodium benzoate (0.02%) was added. Methyl paraben reduced mold development, whereas the sodium benzoate did not. Whether psyllids could be surface sterilized to minimize the introduction of contaminants could be explored.

Survival of adults feeding on the base sucrose solution was marginally reduced for a period of time when the diet contained agar, although at the end of a 2-wk feeding period there was no significant difference in percentage of survival between adults fed the sucrose diet with or without agar. Agar can be added to the base sucrose diet to enhance observations on numbers of feeding sheaths through a membrane and into diet; however, not all potential diet constituents may be compatible with agar, and the presence of agar in the diet confounds pH testing.

Manually stretching the feeding chamber membranes was generally not difficult, although the membranes sometimes tore and had to be replaced. Of concern were unavoidable inconsistencies in the extent the membranes were stretched and whether differences in membrane thinness affected adult feeding. We experimented with the diet encapsulation method due to the probability that the membrane would be more uniformly stretched using the vacuum device. Survival of adults feeding on encapsulated diet was not directly compared with survival of adults in our conventional feeding chambers, however, survival rates generally seemed to be similar (e.g., mean  $\pm$  SEM 14-d daily percentage of survival was  $66.7 \pm 0.0\%$  for adults

feeding on encapsulated diet, compared with  $74.2 \pm 6.6\%$  for adults feeding on diet in the conventional chambers [the latter percentage from data presented in Fig. 1]). Advantages of the encapsulated diet included a faster set up than manually stretching the membrane and problems with tearing were eliminated. A disadvantage of the encapsulated diet was that it was difficult to remove psyllids from individual chambers without psyllids in other chambers escaping. This was because all 24 chambers in a plate were simultaneously covered with the culture plate lid.

Research presented here pertains to a diet for adult *D. citri*, but an artificial rearing system for immatures would be desirable. This system would encourage oviposition. Females feeding on the base sucrose diet in the feeding chambers sometimes oviposited, but few eggs were generally observed. Although on plants females oviposit eggs one by one, in the chambers we sometimes observed strings of eggs, as if a female simply ejected them. There was no consistent oviposition site noted among eggs observed in the chambers. On plants, eggs are attached to the surface of leaves by a slender stalk (0.038 mm long) that is thrust into leaf tissue (Husain and Nath 1927). The feeding chambers studied, in particular the membrane, may have been inadequate with respect to encouraging oviposition and accommodating insertion of these egg stalks.

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