IMPROVED ARTIFICIAL FEEDING SYSTEM FOR REARING THE WHITEFLY *BEMISIA ARGENTIFOLII* (HOMOPTERA: ALEYRODIDAE)

ELIZABETH W. DAVIDSON[1], MICHELLE L. FAY[1], JACQUELYN BLACKMER[2] AND MARK LAVINE[3]

[1]Department of Biology, Arizona State University, Tempe, AZ 85287-1501

[2]USDA/ARS Western Cotton Research Laboratory, Phoenix, AZ 85040

[3]Department of Entomology, University of Wisconsin, Madison, WI 53706

ABSTRACT

The artificial rearing system for *Bemisia argentifolii* Bellows and Perring has been improved by the selection of an autoclavable, reusable membrane, reduction of the sucrose concentration, choice of yeast extract, use of an antifungal agent in the petri dish chamber, choice of surface sterilization agent, egg storage, and maintenance of high humidity in the chamber during the entire nymphal development. We can now produce small numbers of adult whiteflies of both sexes on these chambers, confirm-

ing the utility of these improvements. We also reared two *Bemisia tabaci* A-strain to adults and several *Trialeurodes vaporariorum* nymphs to the 3rd and 4th instar on the improved feeding system.

Key Words**:** artificial diet, *Bemisia tabaci* A-strain, *Trialeurodes vaporariorum*

RESUMEN

El sistema de cría artificial para *Bemisia argentifolii* Bellows y Perring ha sido mejorado tras la selección de una membrana reutilizable y autoclaveable, reducción en concentración de sacarosa, selección de extracto de levadura, uso de un agente antihongo en la cámara de placa petri, selección de agente de esterilización de superficie, almacenaje de huevos, y mantenimiento de alta humedad en la cámara durante el completo desarrollo ninfaceo. Ahora podemos producir números pequeños de moscas blancas adultas de ambos sexos en estas cámaras, confirmando la utilidad de estas mejorías. También criamos dos *Bemisia tabaci* linaje-A con adultos de varias ninfas *Trialeurode vaporariorum* al 3ro y 4to instar bajo el nuevo sistema de alimentación.

We previously reported an artificial system for rearing the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring (= *B. tabaci* B-biotype), to the 3rd instar (Jancovich et al. 1997). This system used a polycarbonate chamber, a Parafilm® membrane, and a filter-sterilized diet consisting of 30% sucrose and 5% yeast extract in distilled water. Although this system has proven useful for gut function studies (E. W. D. & R. Rosell, unpublished) and for assays of potential ingested toxins (Jancovich et al. 1997; E. W. D., unpublished), it requires very careful, aseptic techniques and rarely permits development beyond the 4th instar. Assays using this system often failed due to fungal contamination. Our goals in improving the feeder system and diet were to increase egg hatch, to reduce fungal contamination, and to bring a high proportion of *B. argentifolii* nymphs to 3rd or 4th instar within 14 days, in order to use these nymphs as hosts for parasitic wasps (Davidson & Jones 1999). We also wished to investigate the use of the system for the culture of other whitefly species. We report here improvements in this system that accomplish some of these goals and also enable successful development of a proportion of *B. argentifolii* nymphs to adults.

MATERIALS AND METHODS

Feeder Assembly

Several commercially available, autoclavable filtration membranes were tested to replace the Parafilm® membrane, including MSI® TefSep (Micron Separations-Osmonics Inc., Westboro, MA), Durapore® SVLP (Millipore Inc., Bedford, MA) and Millipore® TCTP membranes with pore sizes of 0.2-10 μm. TefSep PTFE autoclavable filtration membranes, 1.0 μm, 45 mm diameter (MSI-Osmonics) were found to be most acceptable and were used in all further experiments.

The feeder assembly, which consists of a bottom chamber, membrane, and upper plate held together by binder clips (Jancovich et al. 1997), was fully assembled and autoclaved (120°C for 20 min) before filling with the diet solution. Stainless steel 20 mm electrophoresis binder clips ("Father Time Clips", Research Products International, Mt. Prospect, IL) were used.

To inhibit fungal contamination of feeders, the interior of sterile glass or disposable plastic petri dishes was rinsed with a 0.1% solution of miconazole (Sigma, St. Louis, MO) in 95% ethanol which was then permitted to evaporate, leaving a residue of antifungal agent. High humidity was maintained by adding a damp filter paper triangle to each petri dish and by placing a sterile slide, held in place with a bent hairclip, over the diet chamber after eggs were deposited. Sterile, filled feeders were held individually in sterile petri dishes. Assembly took place under a laminar-flow hood with a germicidal ultraviolet lamp, and all equipment was exposed to the ultraviolet light for approximately 30 min before assembly.

Egg Harvest and Treatment

Bemisia argentifolii eggs were harvested from cotton, collards or melon. Leaves were chosen that contained primarily darkened eggs, that were close to hatching. Leaves were dipped sequentially into a detergent solution, distilled water, a 10% household chlorine bleach solution (final concentration 0.5% sodium hypochlorite) for 2-3 min, to loosen the egg pedicel, and distilled water. In some experiments, after the 10% bleach step, leaves were dipped in a 3% solution of sodium thiosulfate to remove residual chlorine, followed by a rinse with distilled water. Eggs were removed using a WaterPik® dental device, filtered through 3 layers of organdy cloth (6-8 fibers/mm) and collected on coffee filters. Eggs were then transferred to sterile 50-ml plastic centrifuge tubes and surface sterilized using 70% ethanol (approximately 1 min) followed by either a 10% bleach solution or a 10% Roccal II® solution (final concentration 1% alkyl dimethyl butyl ammonium chloride, Sterling Drug) (2-3 min). In some experiments, a rinse of 3% sodium thiosulfate solution followed the bleach step, to neutralize residual chlorine. As Roccal is no longer commercially available, the product that has replaced Roccal, Lysol® IC (final concentration 1.1% alkyl dimethyl butyl ammonium chloride, 0.12% didecyl dimethyl ammonium chloride), was also tested, as well as 3% hydrogen peroxide. Eggs were rinsed in sterile distilled water and pipetted onto feeder membranes, then excess water was removed. Assembled feeders were held in sealed plastic boxes with an open water container, on the laboratory bench at 24-25°C and a photoperiod of 10:14 (L:D).

Bemisia tabaci Gennadius (A-strain) eggs were obtained from a colony maintained at the University of California, Riverside. *Trialeurodes vaporariorum* Westwood eggs were obtained from the USDA-ARS Biological Control of Insects Research Unit, Weslaco, TX. Eggs were harvested from leaves and surface sterilized using Roccal as described above.

Storage of Eggs

One cohort of *B. argentifolii* eggs washed from melon leaves was divided into 4 lots and held in water at 4°C for 0, 1, 4, or 7 days. The eggs were then surface sterilized using Roccal and placed on feeders.

Diet Modifications

Yeast extract lots manufactured by Difco (Detroit, MI) and BBL (Becton Dickinson Co., Cockeysville, MD) were compared at 5% concentration. Sucrose concentration was compared at 30% and 15%. Dietary pH was adjusted to pH 5 to 8. The antifungal agents methyl paraben and potassium sorbate (Sigma) were added to the standard diet at concentrations shown to inhibit growth in whitefly diet of fungi isolated from whitefly feeders.

Eggs were counted at day 1 after setup and nymphs were counted by instar at days 5 and 14 (+/- 1 day). Feeders were then held until day 28 to observe adult emergence. Egg sterilization procedures and modifications to the diet were evaluated based upon hatch percentage and percentage of nymphs that had achieved 3rd or 4th instar (including the "red eye" stage) by day 14, based upon total nymphs at day 5. All diet modifications were evaluated in comparison to cohorts reared on standard diet (15% or 30% sucrose, as noted, plus 5% yeast extract). Means and standard deviations were calculated using Excel® 97 SR-2 (Microsoft), and ANOVA followed by Tukey's separation of means was performed using Systat® version 8.0. Means were compared within but not between experiments.

Results and Discussion

The greatest improvement in the rearing technique has resulted from the adoption of an autoclavable, commercially available membrane to replace Parafilm®. TefSep filter membranes are composed of Teflon® (PTFE), are very thin (175 μm), and are hydrophobic. Higher hatch percentages were obtained on PTFE filter membranes than on Parafilm (data not shown). The nymphs attached and fed readily in the oval spaces between the plastic screen fibers that support the membrane. These feeding sites are equally spaced across the membrane surface and occur in parallel rows, which facilitated counting of eggs and nymphs. In preliminary trials, nymphs were unable to feed on membranes with pore sizes of 0.2 or 0.5 μm. The requirement for pores above 0.5 μm is probably related to the cross-section diameter of the stylet bundle. In adult *B. argentifolii*, the stylet bundle is approximately 0.3 μm at the tip and 1.8 μm in cross section closer to the head (Rosell et al. 1995). These results suggest that the nymphs insert stylets through the pores in the filter membrane, but do not puncture the membrane itself. Membranes with pore sizes of 2 μm or larger were unacceptable due to leakage. The MSI TefSep 1 μm membranes are now used in all experiments, and the ability to autoclave the entire feeder system has been a major improvement in reducing microbial contamination. These membranes are, however, significantly more expensive than Parafilm (about $2.00 each).

Ten percent bleach was previously used both to loosen egg pedicels and to surface-sterilize eggs washed from leaves (Jancovich et al. 1997). Rinses of leaves with sodium thiosulfate ($Na_2S_2O_3$) to neutralize chlorine led to decreased egg hatch but did not affect development (Table 1A). Rinses of bleach-treated eggs with sodium thiosulfate did not markedly improve hatch or development to 3rd or 4th instars (Table 1A). Substitution of the antibacterial-antifungal agent, Roccal, for bleach during egg surface-sterilization resulted in a higher percentage of nymphs that reached the 3rd or 4th instar by day 14 (Table 1B). Unfortunately, Roccal is no longer manufactured. The product that is sold as a replacement, Lysol IC, contains didecyl dimethyl ammonium chloride in addition to the active ingredient in Roccal, alkyl dimethyl butyl ammonium chloride. Lysol IC reduced egg hatch and development to 3rd or 4th instars when compared with Roccal (Table 1C). Hydrogen peroxide, shown to be useful in surface sterilization of leafhopper eggs (Wayandande & Fletcher 1998), led to clumping of eggs on the membrane and reduced egg hatch, but development of nymphs that became established was equivalent to that in Roccal and Clorox treatments (Table 1C).

High humidity appears to be essential to development of *B. argentifolii* nymphs on the artificial diet system. Placement of sterile slides over diet chambers to maintain high humidity within the chambers significantly improved development of nymphs to 3rd or 4th instars (Table 1D). Leaf osmotic potential was similarly found to influence hatch and survival of greenhouse whitefly eggs Castañé & Savé 1993). However, all water must be removed from the eggs after deposition on the membrane, as even a small amount of liquid water inhibits egg (hatch.

TABLE 1. EFFECTS OF EGG STERILIZATION PROCEDURES, AND ADDITION OF STERILE SLIDES TO GROWTH CHAMBERS ON HATCH PERCENTAGE AND NYMPHAL DEVELOPMENT. PERCENTAGE OF NYMPHS ACHIEVING 3RD OR 4TH INSTAR BY DAY 14 WAS BASED UPON TOTAL NYMPHS AT DAY 5. STANDARD DIET (5% DIFCO YEAST EXTRACT IN 15% SUCROSE, PH 7.0), 24-25°C (UNLESS OTHERWISE NOTED), PHOTOPERIOD 10:14 (L:D). MEANS ARE COMPARED WITHIN EACH EXPERIMENT. MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT (ANOVA, $\alpha = 0.05$).

Treatment	Mean % egg hatch, day 5 (+/- S.D.)	Mean % 3-4 instars, day 14 (+/- S.D.)	Total nymphs, day 5 (total feeders)
A. sodium thiosulfate neutralization of chlorine			
$Na_2S_20_3$ rinse	51.0 (2.6) a	27.6 (6.5) a	1349 (7)
No rinse	61.8 (5.3) b	26.4 (3.9) a	1084 (7)
B. egg sterilization			
Clorox	61.0 (8.1) a	36.5 (8.1) a	771 (6)
Clorox + $Na_2S_20_3$	56.9 (2.5) a	39.5 (5.2) a,b	633 (6)
Roccal	59.5 (5.2) a	49.0 (7.5) b	596 (6)
C. egg sterilization			
Hydrogen peroxide	24.3 (5.0) a	34.1 (3.3) a	333 (6)
Lysol IC	42.4 (5.7) b	27.1 (3.0) b	575 (6)
Roccal	54.7 (4.1) c	34.2 (4.8) a	924 (6)
D. addition of sterile slides to chambers			
Slides	54.2 (4.5) a	42.3 (5.6) a	1175 (7)
No slides	55.6 (5.1) a	33.6 (2.8) b	1327 (7)

Eggs washed from leaves, but not surface-sterilized, can be stored in water at 4°C for at least one day with no reduction in egg hatch (Fig. 1A) or development to 3rd or 4th instars (Fig. 1B). Leaves bearing eggs can also be stored at least one day under refrigeration (data not shown). These procedures facilitate setup of experiments, as egg harvest can be done at least one day in advance.

Difco and BBL yeast extract produced similar results (Table 2A, B). One lot of yeast extract from Sigma did not produce any 3rd or 4th instar nymphs by day 14 (data not shown). Difco yeast extract that had been stored at room temperature for more than 3 years was significantly less effective in maintaining whitefly growth than fresh yeast extract (Table 2A, B). Yeast extract is now purchased in small quantities and stored in a dessicator at room temperature or at –20°C.

Increasing the concentration of yeast extract in the diet from 5% to 7.5% resulted in lower egg hatch, but did not affect development to 3rd or 4th instars (Table 2B). The diet used in our original study (Jancovich et al. 1997) contained 30% sucrose, based upon analysis of phloem sap. Reduction of sucrose concentration to 15%, however, had slight or no adverse effect on nymphal development and resulted in greater egg hatch (Table 2C, D). Egg hatch was greater on TefSep membranes than on Parafilm membranes (data not shown). These results taken together suggest that water from the diet may evaporate slowly through the porous membranes, contributing to higher humidity around the eggs.

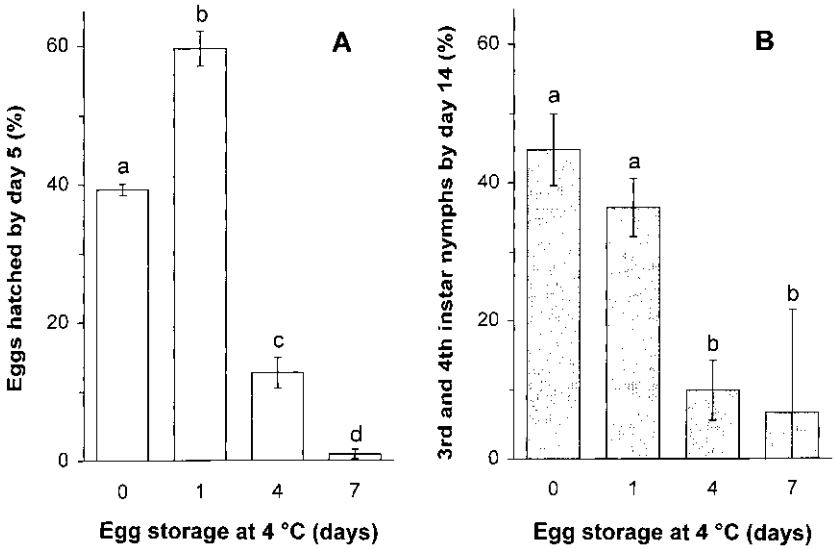

Fig. 1. Effect of storage of eggs in water for 0, 1, 4 or 7 days at 4°C, on A) hatch rate and B) development. Percentage of nymphs achieving 3rd or 4th instar at day 14 was based upon total nymphs at day 5. Standard diet (5% Difco yeast extract in 15% sucrose, pH 7.0), 24-25°C, photoperiod 10:14 (L:D). Bars with the same letter are not significantly different (ANOVA, α= 0.05).

Diet pH did not significantly affect egg hatch. Developmental response was variable, but pH 6.5 and 8.0 diets supported the highest percentage of nymphs to the 3rd and 4th instar (Table 2E). A pH of 5.0 failed to support development to the 3rd or 4th instar (data not shown). Salvucci et al. (1997) found that in adult whiteflies the optimum pH for ingestion of a 20% sucrose diet was between 6.5 and 7.5, in a tested range of 4.5 to 8.5.

The antifungal agent methyl paraben (p-hydroxybenzoic acid methyl ester), +/- potassium sorbate, added to the diet at concentrations effective in inhibiting fungi, was strongly inhibitory to both hatching and nymphal development (Table 2F). These agents were apparently toxic to eggs and may have acted as antibiotics against the symbiotic microorganisms that are necessary for whitefly development (Costa et al. 1997). The reduction of egg hatch due to addition of these compounds to the diet also implies that dietary components other than water may make contact with the eggs through the membranes. Eggs did not hatch when miconazole was used as an egg rinse (data not shown). However, miconazole residue in the petri dishes holding the feeders was beneficial in inhibiting fungal development in the petri dish chambers.

It is difficult to compare survivorship on artificial diet with that observed on plants, since predation, parasitism, disease, plant quality and weather conditions are not factors in mortality of artificially reared nymphs. On plants, survival from eggs to adults can range from approximately 40% to over 80% (Horowitz et al. 1984, Powell & Bellows 1992, Wagner 1995). Although production of adult whiteflies on the artificial diet was not the goal of this study, we observed emergence of adult whiteflies of both sexes by 28 days equivalent to 0.5% to 2% of the total nymphs counted at day 5. The highest percentages of *B. argentifolii* adults were produced when Roccal was used to sterilize eggs, slides were added to chambers, and sucrose was reduced to 15%.

TABLE 2. DIETARY ALTERATIONS. "NEW" = STORED LESS THAN 1 YEAR; "OLD" = STORED MORE THAN 3 YEARS. PERCENTAGE OF NYMPHS ACHIEVING 3RD OR 4TH INSTAR AT DAY 14 WAS BASED UPON TOTAL NYMPHS AT DAY 5. PH 7.0 UNLESS OTHERWISE NOTED, 24-25°C, PHOTOPERIOD 10:14 (L:D) OR *28°C 14:10 (L:D). MEANS ARE COMPARED WITHIN EXPERIMENTS. MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT (ANOVA, $\alpha = 0.05$).

Yeast extract manufacturer, %, age; sucrose %	Mean % egg hatch, day 5 (+/- S.D.)	Mean % 3-4 instars, day 14 (+/- S.D.)	Total nymphs, day 5 (total feeders)
A. age of yeast extract			
Difco, 5, old; 30	59.5 (5.4) a	28.3 (5.9) a	1327 (6)
Difco, 5, new; 30	61.0 (9.0) a	43.1 (4.9) b	1098 (6)
BBL, 5, new; 30	58.7 (4.8) a	39.5 (8.0) b	1208 (6)
B. percent yeast extract			
Difco, 5, new; 30	44.1 (4.9) a	58.0 (9.6) a	388 (6)
Difco, 7.5, new; 30	35.4 (6.3) b	46.8 (6.0) a	208 (5)
C. percent sucrose, experiment 1			
Difco, 5, new; 30	27.0 (5.2) a	47.0 (6.3) a	388 (8)
Difco, 5, new; 15	57.6 (7.0) b	32.7 (6.0) b	1072 (10)
D. percent sucrose, experiment 2			
*BBL, 5, new; 30	40.1 (4.1) a	37.2 (9.1) a	701 (6)
*BBL, 5, new; 15	60.6 (12.1) b	32.6 (6.0) a	1184 (7)
E. diet pH			
*BBL, 5, new; 15; pH 6.5	69.2 (11.8) a	33.8 (3.6) a,b	964 (7)
*BBL, 5, new; 15; pH 7.0	66.1 (6.6) a	27.0 (2.6) a	1157 (7)
*BBL, 5, new; 15; pH 7.5	57.0 (8.7) a	25.0 (7.0) a	859 (7)
*BBL, 5, new; 15; pH 8.0	56.0 (11.1) a	40.2 (11.9) b	772 (7)

TABLE 2. (CONTINUED) DIETARY ALTERATIONS. "NEW" = STORED LESS THAN 1 YEAR; "OLD" = STORED MORE THAN 3 YEARS. PERCENTAGE OF NYMPHS ACHIEVING 3RD OR 4TH INSTAR AT DAY 14 WAS BASED UPON TOTAL NYMPHS AT DAY 5. PH 7.0 UNLESS OTHERWISE NOTED, 24-25°C, PHOTOPERIOD 10:14 (L:D) OR *28°C 14:10 (L:D). MEANS ARE COMPARED WITHIN EXPERIMENTS. MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT (ANOVA, $\alpha = 0.05$).

Yeast extract manufacturer, %, age; sucrose %	Mean % egg hatch, day 5 (+/- S.D.)	Mean % 3-4 instars, day 14 (+/- S.D.)	Total nymphs, day 5 (total feeders)
F. antifungal agents			
Difco, 5, new; 15	45.5 (4.0) a	39.7 (1.8) a	1367 (6)
Difco, 5, new; 15; 0.1% methyl paraben	11.9 (2.9) b	27.9 (6.1) b	300 (5)
Difco, 5, new; 15; 0.1% methyl paraben + 0.1% potassium sorbate	11.0 (2.2) b	11.4 (6.6) c	347 (6)

TABLE 3. DEVELOPMENT OF *BEMISIA TABACI* A-STRAIN AND *TRIALEURODES VAPORARIORUM* ON THE ARTIFICIAL FEEDING SYSTEM. PERCENTAGE OF NYMPHS ACHIEVING 3RD OR 4TH INSTAR AT DAY 14 WAS BASED UPON TOTAL NYMPHS AT DAY 5. STANDARD DIET (5% DIFCO YEAST EXTRACT, 15% SUCROSE, PH 7.0), 24-25°C, PHOTOPERIOD 10:14 (L:D).

Mean % egg hatch, day 5 (+/- S.D.)	Mean % 3-4 instars, day 14 (+/- S.D.)	Total nymphs, day 5 (total feeders)
	Bemisia tabaci A-strain	
19.8 (1.7)	26.0 (7.7)	271 (6)
	Trialeurodes vaporariorum, experiment 1	
22.3 (9.8)	4.7 (2.1)	84 (3)
	Trialeurodes vaporariorum, experiment 2	
31.2 (13.8)	3.7 (6.4)	78 (3)

Twenty-six percent of *Bemisia tabaci* (A-strain) developed to 3rd or 4th instar within 14 days and two adult whiteflies emerged after 28 days on standard diet (5% Difco yeast extract, 15% sucrose) (Table 3). Although percent hatch and development for the A-strain whitefly was lower than that normally observed with *B. argentifolii*, these results suggest that the feeder system is adequate for the development of *B. tabaci* A-strain whiteflies, and may prove useful in investigating the physiological differences between these closely-related species. The greenhouse whitefly, *T. vaporariorum,* hatched and began to feed on the artificial diet system, but only a small number developed to the 3rd or 4th instar by 14 days (Table 3). Nonetheless, development of even a few *T. vaporariorum* nymphs on the *Bemisia* artificial diet suggests that this diet could provide the basis for a diet for the greenhouse whitefly. Short-term bioassay of ingested compounds against greenhouse whitefly nymphs is possible using this assay system, similar to the plant tissue culture system used by Melé et al. (1992).

ACKNOWLEDGMENTS

We are grateful to C. LaVesque, T. Perring and W. Jones for providing eggs of *B. tabaci* A-strain and *T. vaporariorum,* and to L. Lee for technical assistance. This research was supported by USDA CSREES 9702182 and a Cooperative Agreement with the USDA-ARS Biological Control of Insects Research Unit, Weslaco, TX.

REFERENCES CITED

CASTAŇÉ, C., and R. SAVÉ. 1993. Leaf osmotic potential decrease: a possible cause of mortality of greenhouse whitefly eggs. Entomol. Exper. Appl. 69: 1-4.

COSTA, H. S., T. J. HENNEBERRY, AND N. C. TOSCANO. 1997. Effects of antibacterial materials on *Bemisia argentifolii* oviposition, growth, survival, and sex ratio. J. Econ. Entomol. 90: 333-339.

DAVIDSON, E. W., AND W. JONES, 1999. Successful rearing of parasitoid wasps on *Bemisia argentifolii* cultured on artificial diet. Silverleaf Whitefly, National Research, Action and Technology Transfer Plan, 1997-2001, Second Annual Review of the Second 5-Yr Plan. USDA, ARS 1999-01, p. 69.

HOROWITZ, A. R., H. PODOLER, AND D. GERLING. 1984. Life table analysis of the tobacco whitefly *Bemisia tabaci* in cotton fields in Israel. Acta Oecol. Applic. 5: 221-233.

JANCOVICH, J. K., E. W. DAVIDSON, M. LAVINE, AND D. L. HENDRIX. 1997. Feeding chamber and diet for culture of nymphal silverleaf whitefly, *Bemisia argentifolii*. J. Econ. Entomol. 90: 628-633.

MELÉ, E., J. MESSEGUER, R. GABARRA, J. TOMÁS, J. COLL, AND F. CAMPS. 1992. *In vitro* bioassay for the effects of *Ajuga reptans* phytoecdysteroids on *Trialeurodes vaporariorum* larval development. Entomol. Exp. Appl. 62: 163-168.

POWELL, D. A. AND T. S. BELLOWS, JR. 1992. Preimaginal development and survival of *Bemisia tabaci* on cotton and cucumber. Environ. Entomol. 21: 359-363.

ROSELL, R.C., J. E. LICHTY, AND J. K. BROWN. 1995. Ultrastructure of the mouthparts of adult sweetpotato whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae). Int. J. Insect Morphol. & Embryol. 24: 297-306.

SALVUCCI, M. E., G. R. WOLF, AND D. L. HENDRIX. 1997. Effect of sucrose concentration on carbohydrate metabolism in *Bemisia argentifolii*: Biochemical mechanism and physiological role for trehalulose synthesis in the silverleaf whitefly. J. Insect Physiol. 43: 457-464.

WAGNER, T. L. 1995. Temperature-dependent development, mortality, and adult size of sweetpotato whitefly Biotype B on cotton. Environ. Entomol. 24: 1179-1188.

WAYANDANDE, A. C. AND J. FLETCHER. 1998. Development and use of an established cell line of the leafhopper *Circulifer tenellus* to characterize *Spiroplasma citri*-vector interactions. J. Invertebr. Pathol. 72: 126-131.