A Novel Liquid Larval Diet and Its Rearing System for Melon Fly, *Bactrocera cucurbitae* (Diptera: Tephritidae)

CHIOU LING CHANG,[1] CARLOS CACERES,[2] AND ERIC B. JANG[3]

Ann. Entomol. Soc. Am. 97(3): 524–528 (2004)

ABSTRACT A liquid larval diet and its rearing system for *Bactrocera cucurbitae* Coquillett fruit fly production were developed. The diet was composed of brewer's yeast, sugar, antifungal agents (sodium benzoate and nipagen), citric acid, and distilled water. Sponge cloth placed in rearing trays was used as a support substrate for larvae, alleviating the need for the traditional (mill feed) bulking agent. Larval rearing of *B. cucurbitae* on this diet resulted in ≈20% less pupal production and ≈10% lighter pupal weight than from the control diet, whereas pupal density, adult emergence, adult fliers, and egg hatch showed no significant discrepancies. Pupal recovery increased with yeast concentrations up to 14.2%. Benefits derived from a liquid diet include reduction in postrearing waste, alleviation of (pesticide-free) bulking agent, and reduction in diet ingredient storage and labor. These benefits must be weighed against any reductions in production and size when large-scale mass rearing of fruit flies for use in sterile insect release programs are evaluated.

KEY WORDS liquid diet, fruit fly diet, *Bactrocera cucurbitae*

REARING OF THE MELON FRUIT FLY, *Bactrocera cucurbitae* Coquillett, by using a liquid diet has been a long-term objective for tephritid fruit fly mass-rearing programs over the past three decades. Because melon flies have mouthparts that serve dual functions (sucking or piercing-and-sucking), the benefits obtained by conversion from a solid bulk agent-based diet to a liquid diet have several operational advantages. One major problem with conversion to an all-liquid diet is finding an optimal substrate for the development of first and second instars. Delivery substrates, such as agarose for the English grain aphid, *Sitobion avenae* (F.) (Urbanska et al. 1998), cotton for the parasitoid *Exorista larvarum* (L.) (Mellini et al. 1996), paper for the lady beetle *Epilachna vigintioctopunctata* (F.) (Murata et al. 1994), acrylamid-acrylate polymer gelling agent (water-lock G-400) for screwworm, *Cochlimyia hominivorax* (Coquerel) (Taylor and Mangan 1987, Taylor et al., 1991), or cotton toweling for the olive fruit fly *Bactrocera oleae* (Gmelin) (Mittler and Tsitsipis 1973), have been used for insect liquid diets. However, high mortality and low yield have been reported in some studies (Halanda 1976, Brown and Snow 1978, Tsitsipis and Kontos 1983, Ochieng et al. 1987, Letardi and Caffarelli 1990). The wheat-bran–based fruit fly larval diet currently used in USDA–ARS Pacific Basin Agricultural Research Center in Honolulu has performed well for >30 yr, and, except for minor changes (primarily in bulking agents), is used worldwide for mass rearing of tephritid fruit flies, including melon fly (Tanaka et al. 1969). This diet contains bran or wheat products as bulking agents that provide young larvae with a support substrate, as well as some nutritional value. However, wheat-based bulking agents such as the bran also have some problems associated with use, including inconsistency of quality and water absorbency, pesticide contamination, spent (used) diet management (disposal and tray cleaning), storage space, high costs, labor, and sanitation. In some fruit fly mass-rearing programs, wheat-based products have been replaced by sugarcane bagasse (Vargas et al. 1983) and toilet paper (Kakinohana and Yamagishi 1991). However, these replacements suffer from many of the same problems (especially storage disposal and cleaning) as the wheat-based bulking agents. As mass production of tephritid fruit flies for use in the sterile insect technique (SIT) has increased, production facilities have had to take a very close look at the costs of production and rearing efficiency as part of their overall operations. Ideally, a liquid-based diet with a recyclable substrate system would alleviate many of the problems listed above and reduce the overall costs of production. With these objectives in mind, we developed a simple liquid diet and a cost-efficient rearing system for *B. cucurbitae* fruit fly rearing.

Materials and Methods

Insects. Melon fly eggs were obtained from a laboratory colony at the Pacific Basin Agricultural Research Center. This laboratory colony was reared on a wheat-based artificial diet (Tanaka et al. 1969) mill

[1] U.S. Pacific Basin Agricultural Research Center, USDA–ARS, 2727 Woodlawn Dr., Honolulu, HI 96822.
[2] Entomology unit, FAO/IAEA Agriculture and Biotechnology Laboratory, A-24444, Seibersdorf, Austria.
[3] U.S. Pacific Basin Agricultural Research Center, USDA–ARS, P.O. Box 4459, Hilo, HI 96720.

0013-8746/04/0524–0528$04.00/0

Table 1. Ingredients for a novel liquid diet and currently used mill feed diet recipe for larvae of *B. cucurbitae*

Diet	Liquid diet		Mill feed diet	
Ingredients	%	g	%	g
Sodium benzoate	0.09	0.11	0.11	0.11
Nipagen	0.09	0.11	0.11	0.11
Sugar	5.96	7.35	7.35	7.35
Torula yeast			3.55	3.55
Brewer's yeast	11.51	14.20		
Mill feed			31.19	31.19
Water	81.08	100.00	57.70	57.70
Citric acid	1.26	1.56		

feed diet for >360 generations (Table 1). Eggs were collected within an hour and kept in an room (25°C, 65% RH, photoperiod of 12:12 [L:D] h) to maintain constant developmental conditions until used.

Diet Formulation. The liquid diet developed for *B. cucurbitae* was composed of brewer's yeast (gift from Carlos Caceres, FAO/IAEA, Agriculture and Biotechnology Laboratory, Seibersdorf, Austria), sugar, antimicrobial agents (sodium benzoate and nipagen), citric acid, and water (Table 1). The mill feed diet formulation currently used at PBARC served as the control diet in this study (Table 1). Initial pH values for these diets were between 3.3 and 3.8.

Diet Preparation and Delivery System. The diet mixture is formulated by weighing all the above-mentioned ingredients into a 250-ml polyethylene container (with lid) and either shaking it vigorously by hand to mix homogeneously or using a magnetic stirrer and mixing plate for ≈5 min or until the diet ingredients were fully dissolved and homogeneous. Sponge cloth (31.5 by 25.7 cm, Kalle USA Inc., Flemington, NJ) was the primary support matrix for feeding larvae. Each side of the sponge cloth has a different surface pattern: one side is a diamond-shaped pattern surface and the other is patterned with grooves. In this study, the groove side was presented on the top surface of the rearing tray. In this study, one piece of sponge cloth and polyethylene screen (Home Depot, Aiea, HI) was layered on the floor of a larval tray (18 by 12 by 3.5 cm^3, Premium Incorporated, Honolulu, HI) (sponge cloth on the top of screen). The diamond-shaped pattern surface was facing down adjacent to the polyethylene screen underneath. The sponge material was 1.5 cm away from each of the four sides of larval tray. One hundred milliliters of the liquid diet was then poured over the sponge cloth to saturate the cloth and to allow extra liquid to flow over the floor of the larval tray to maintain the moisture and food source but not cover the sponge cloth. This provided a suitable substrate for the developing larvae, especially for the first and second instars.

Experimental Procedures. Eggs (1 ml, ≈25,000 eggs per milliliter) were seeded using a 1-ml transfer pipette onto a strip of wet sponge cloth (2 by 7 cm) placed on the center top surface of the sponge cloth of the larval tray containing freshly made liquid diet. Four trays were used for every test (≈1 million eggs). Upon hatching from the egg case, larvae fed ad libitum on the liquid diet at 25°C, 65% RH for 5 d. By occasional observation, we observed younger larvae were usually found on the top surface of the sponge, whereas older (late second and third instars) were partially submerged under the sponge cloth, screen, or between sponge cloth and screen until ready to pupariate. After 5 d, trays containing larvae were then transferred to 20–21°C, 65% RH for pupariation and to slow down yeast fermentation that had built up during the previous 5 d. Ten days after seeding eggs on the tray, pupae were collected using a strainer and placed in containers containing corncob grits or vermiculite as a pupation substrate. Pupal production was expressed as weights (grams) and volumes (milliliters) of total collected pupae, and pupal recovery was calculated based in the initial numbers of eggs seeded on the diet. Numbers of pupae were estimated either by the volume or weight. Eight groups of 100 pupae each were also weighed to compare pupal weight with those of pupae reared from the ARS mill feed diet (control). Collected pupae were then set up for flight ability test according to the description of Boller et al. (1981) and Chang et al. (2001).

Linolenic Acid. Based on the information from NRC (1982) and the melon fly diet currently in use (Table 1), mill feed contains 16.5 times more total fatty acids than brewer's yeast. Therefore, two doses of linolenic acid (0.2 and 0.4 ml per recipe) were added to the liquid diet to compensate for the replacement of fatty acids in the mill feed and to evaluate the effect of these two doses of exogenous fatty acid on pupal recovery. We used linolenic acid for this study because unsaturated fatty acids comprised a large portion of fatty acids in both mill feed and brewer's yeast, and it was inexpensive and easily available.

Statistical Analysis. The evaluation of the liquid diet and its delivery system was based on larval duration, pupal recovery, pupal weight, adult emergence, adult flight ability, and egg hatch as described by Chang et al. (2001). Each diet batch had four replicated trays for each treatment, and data were averaged to obtain mean values. At least four different diet batches were replicated using the same treatment. Differences in the diet batch/treatment data were determined by analysis of variance (ANOVA), with the honestly significant difference value calculated as Tukey's statistic at $\alpha = 0.05$ (SAS Institute 2001).

Results and Discussion

Liquid diet ingredients and their relative amounts for *B. cucurbitae* larvae were based initially on the ARS mill feed recipe (Table 1), excluding torula yeast and bulking agent (mill feed) and replacing their equivalent combined amount with brewer's yeast (34.74% = 3.55% from torula yeast + 31.19% from mill feed) (Table 1). In the beginning, pupal production from the above-mentioned modified recipe was low compared with that from the control mill feed diet (30.38 ± 2.94%; $P < 0.0001$). We then compared the nutrient profiles between brewer's yeast and mill feed according to information from NRC (1982) and mod-

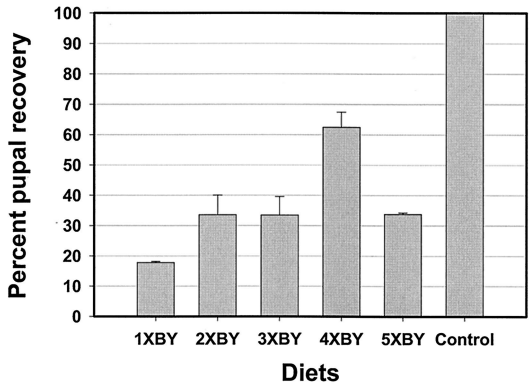

Fig. 1. Mean percentage of pupal recovery ratio from liquid diet-reared larvae of *B. cucurbitae* to those from the control diet. Various doses of brewer's yeast (3.55, 7.10, 10.65, 14.20, and 17.75%) were expressed as 1XBY, 2XBY, 3XBY, 4XBY, and 5XBY, respectively. Currently used ARS mill feed diet was used as control. Bars are represented as standard errors.

ified the amounts used for subsequent evaluations. We concluded that nutrients in brewer's yeast, such as vitamins and amino acids, are ≈3.5-fold more than those in mill feed. Because the nutrients from torula yeast are about equal to those from brewer's yeast and the amount of mill feed (31.19%) in ARS diet formula is ≈10-fold the amount of torula yeast (3.55%) in the ARS diet recipe (Table 1), three-fold more yeast was required to substitute mill feed with yeast. Therefore, total yeast in the liquid diet was increased four-fold from the original (to 14.20%, 4 times brewer's yeast) (Fig. 1). Pupal recovery subsequently increased to ≈60% (Fig. 1) but < 80% when the amount of yeast increased to 14.2% because it was done in the early stage of this experiment. The purpose for this figure is to indicate the best dose of brewer's yeast in the diet. With a constant amount of sugar in the diet, we determined that 14.20% brewer's yeast demonstrated the best pupal recovery. Doses higher than 14.20% resulted in decreased production (Fig. 1).

Larvae reared on the liquid and control diets developed at approximately the same rate. Our results differ from those reported by Fay and Wornoayporn (2002), who found that *Ceratitis capitata* (Wiedemann) larval duration on diet with either high-density or low-density sheet sponge was extended. In our study, newly emerged larvae migrated from the seeded strip randomly and had a tendency to aggregate inside each groove (Fig. 2A). By the fourth day, most of the larvae had started to feed on the surface of sponge cloth and could be found in the liquid portion of the diet or underneath the sponge cloth on the fifth day (Fig. 2B and C). They were ready to pupariate at the sixth day (Fig. 2D). Pupariation occurred on day 7.

Pupal recovery from larvae reared on the liquid diet was significantly lower than that from the control diet (Table 2). Pupal production from liquid diet was ≈80% of the control. This number was different from that reported by Mittler and Tsitsipis (1973) on olive fruit fly, who found a 10-fold increase in pupal yield from 1 g of liquid diet in comparison with those from solid diet. However, we believe pupal recovery can be further improved. The main purpose of this study is to show that *B. cucurbitae* larvae were able to develop and survive in a liquid diet.

Pupal density was calculated using the total pupal weight (grams) divided by the total pupal volume (milliliters). There was no significant difference between the pupal density obtained from either the liquid diet or the control diet ($F = 1.10$; df = 3, 28; $P = 0.3865$). It confirmed that pupal production from both diets is relatively proportional to each other, whether measured by weight or volume.

Pupal weight of 100 pupae from larvae reared on the liquid diet was lighter than that from the control diet (Table 2). Adult emergence from immatures reared on liquid diet was not lower than that from the control diet. No significant differences in flight ability or egg hatch between the liquid diet-reared flies or the control (mill feed diet-reared flies) was observed (Table 2).

Of particular interest was the finding that the amount of fatty acid in brewer's yeast seems to be enough for larval development. Exogenous fatty acid did not increase pupal recovery, pupal weight, flight ability, egg hatch, or adult emergence. In fact, pupal recovery decreased with increasing concentrations of linolenic acid (Table 2). However, evaluation of fecundity from liquid diet-reared flies may be needed to further confirm the importance of fatty acids in this liquid diet.

Larval diets currently in use worldwide for mass rearing of tephritid fruit flies such as the melon fly 1) use large amounts of biological bulking agents such as corncob, bran, mill feed, toilet paper, or bagasse; 2) are labor-intensive in production setup and disposal; and 3) require considerable storage space for bulk ingredients. As a result, management of diet ingredients and spent diet has been a serious problem for fruit fly rearing. Although there are several ways to manage spent diet, they are not cost-efficient. With this liquid diet, there is no spent diet problem.

The delivery matrix, sponge cloth, is lightweight, highly water absorbent, easy to clean, and reusable. These sponge cloths are produced using recycled raw materials (natural cellulose and cotton fibers) and can be disposed of without any problems. They will biodegrade and rot naturally in addition to being lightweight, water absorbent (40 times their weight), easy to clean (washing machine or by hand), and reusable. Diet preparation in the current mass-rearing system is labor-intensive. It starts with weighing the ingredients, pouring them into a large mixer to mix wet and dry ingredients, dispensing the diet into larval trays, seeding the eggs onto the diet, and finally transporting into the appropriate rearing room for incubation and development. Workers have to lift each larval tray weighing ≈10 kg. In contrast, the liquid diet can be mixed in a blender because most of the ingredients are in powder form and water-soluble. The larval tray is

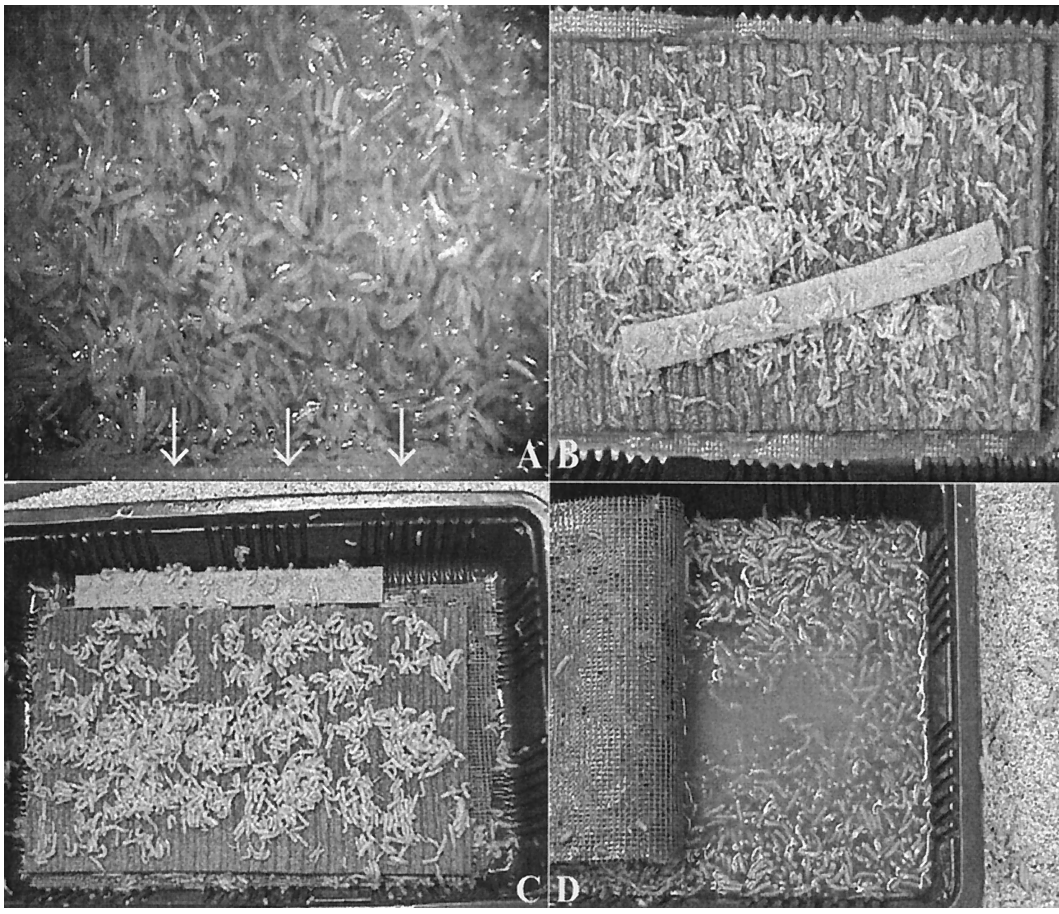

Fig. 2. *B. cucurbitae* larvae were reared on liquid diet. (A) Two-day-old larvae spread out from the seeding strip (↓) toward grooves on the surface of sponge cloth. (B) Four-day-old larvae fed mostly on the top surface of sponge cloth. (C) Five-day-old larvae distributed on the top and bottom of sponge cloth. (D) Six-day-old larvae on the bottom of sponge cloth and ready to pupate (→).

lightweight because of the texture of delivery matrix (sponge cloths). Labor and space saving can be further enhanced by automation. All used sponge cloths can be recycled and washed in the washer or by hand with dishwasher detergent. Environment-controlled storage space for diet ingredients, especially those needing "special care," would be greatly reduced because the lightweight, recyclable sponge cloths replace the "bulky" bulking agent. Incubation space is also saved because of the reduced size of the larval trays. The threats to workers and the environment from acidulation and fermentation of the diet with the current rearing system may decrease. The overall expense of spent diet management, bulking agent replacement, labor saving, and storage space saving would be reduced (Table 3). Moreover, with the basic

Table 2. Comparison of pupal recovery, pupal weight, adult emergence, flier, and egg hatch among *B. cucurbitae* reared on liquid diet with various doses of linolenic acid and control diet

Parameters	Liquid diet with linolenic acid			Control diet	Analysis of variance
	0 ml	0.2 ml	0.4 ml		
Pupal recovery[a] (ml)	65.25b	56.75b	27.25c	83.88a	$F = 30.96$; df = 3, 28; $P < 0.0001$
Pupal weight/100 (g)	1.08b	1.08b	1.07b	1.16a	$F = 21.27$; df = 3, 28; $P < 0.0001$
Adult emergence (%)	86.17a	87.61a	88.11a	82.41a	$F = 0.29$; df = 3, 12; $P < 0.8331$
Flier[b] (%)	80.94a	83.35a	86.28a	79.72a	$F = 0.49$; df = 3, 12; $P < 0.6954$
Egg hatch (%)	93.25a	92.00a	90.00a	87.00a	$F = 1.81$; df = 3, 12; $P < 0.1984$

Within a row, means followed by the same letter are not significantly different ($\alpha = 0.05$; ANOVA test).

[a] Pupal recovery in milliliter was calculated based in the initial numbers of eggs seeded on the diet.

[b] Percentage of flier refers to total number of adults that flew out of the flight testing tube (20 cm) from 100 pupae (Chang et al. 2001).

Table 3. Estimated comparison of liquid and mill feed diets for *B. cucurbitae* on cost and storage space

Ingredients	Liquid diet		Mill feed diet	
	Cost/15 trays ($)	Storage space (cm^2)	Cost/15 trays ($)	Storage space (cm^2)
Mill feed	0.00	0	14.02	55,118.00
Torula yeast	0.00	0	11.62	5120.64
Brewer's yeast	8.96	5120.64	0.00	0
Sugar	5.34	4826.00	5.34	4826.00
Sodium benzoate	0.20	906.78	0.20	906.78
Nipagen	0.61	1219.20	0.61	1219.20
Citric acid	2.20	23,749.00	0.00	0
Sponge cloth	15.75	71.12	0.00	0
Total	33.06	35,892.74	31.79	67,190.62

knowledge and technology learned from this liquid diet, this technology may be applied to rearing other insects such as rearing parasitoid of fruit flies.

In terms of cost and space, a comparison is presented in Table 3 for the production of 15 trays. The cost to make a liquid diet for 15 trays is $1.27 more than the mill feed diet. Cost quoted here is as a small-volume purchase. Bulk purchase of sponge cloth, brewer's yeast, and citric acid should be much less. However, the savings on storage space would be 46.5% of the current space. These saving will however need to be considered relative to the ≈20% reductions in yield based on the current data.

In conclusion, this study has shown that melon fly larvae can be reared on liquid diet with an appropriate delivery system. The advantage of this liquid diet is to eliminate (or obviate) need to use a starter diet as described in Fay and Wornoayporn (2002) and a bulking agent. It also promotes a savings in labor and storage space. There are some concerns that need to be considered: the critical stage for larval survival is within 3 d after hatching. Care must be taken to ensure that the liquid diet does not flood the sponge cloths, causing larval drowning but yet provide sufficient diet for larval development. To meet this requirement, diet can be replenished with fine spray system in a timely manner.

Acknowledgments

We thank Dennis Gonsalves for encouragement and support to submit for patenting. We also thank Danny Kawamoto and Keith Shingetani, USDA–ARS–PBARC rearing unit, for patience and timely supply of fruit flies. Appreciation also goes to IAEA for providing the brewer's yeast and to Drs. Allen Cohen, Muhammad Chaudhury, and Todd Shelly for critical review.

References Cited

Boller, E. F., B. I. Katsoyannos, U. Remund, and D. L. Chambers. 1981. Measuring, monitoring, and improving the quality of mass-reared Mediterranean fruit flies, *Ceratitis capitata* Wiede. 1. The RAPID quality control system for early warning. Z. Angew. Entomol. 92: 67–83.

Brown, H. E., and J. W. Snow. 1978. Protein utilization by screwworm larvae (Diptera: Calliphoridae) reared on liquid medium. J. Med. Entomol. 14: 531–533.

Chang, C. L., R. Kurashima, and C. P. Albrecht. 2001. Larval development of *Ceratitis capitata* (Diptera: Tephritidae) on a meridic diet. Ann. Entomol. Soc. Am. 94: 433–437.

Fay, H.A.C., and V. Wornoayporn. 2002. Inert reusable substrates as potential replacements for wheat bran in larval diets for Mediterranean fruit fly, Ceratitis capitata (Wiede.) (Dipt, Tephritidae). J. Appl. Entomol. 126: 92–96.

Halanda, S. 1976. Use of a liquid artificial diet as a médium for testing systemic insecticidas. Ustav Experimentalnej fytopatologie a Entomologie SAV, Ivanka pri Dunaji, Czechoslovakia. 12: 225–232.

Kakinohana H., and M. Yamagishi. 1991. The mass production of the melon fly: techniques and problems. *In* Proceedings of the International Symposium on the Biology and Control of Fruit flies, K. Kawasaki, O. Iwahashi, and K. Y. Kaneshiro [eds.], FFTC, Ginowan, Okinawa, Japan.

Letardi, A., and V. Caffarelli. 1990. Influence of the utilization of liquid artificial larval diet for rearing *Chrysoperla carnea* Steph. Planipennia Chrysopidae. Redia 73: 79–88.

Mellini, E., G., Campadelli, and M. L. Dindo. 1996. Actual possibilities of mass production of the parasitoid *Exorista larvarum* (L.) (Diptera: Tachinidae) on oligidic diets. Bollettino dell'Instituto di Entomologia Guido Grandi della Universita degli Studidi Bologna 50: 233–241.

Mittler, T. E., and J. A. Tsitsipis. 1973. Economical rearing of larvae of the olive fruit fly, *Dacus oleae*, on a liquid diet offered on cotton toweling. Entomol. Exp. Appl. 16: 292–293.

Murata, M., K. Iwabuchi, and J. Mitsuhashi. 1994. Partial rearing of a phytophagous lady beetle, *Epilachna vigintioctopunctata* (Coleoptera: Coccinellidae). Appl. Entomol. Zool. 29: 116–119.

[NRC] National Research Council. 1982. United States-Canadian Tables of Feed Composition. National Acad. Press, Washington, DC.

Ochieng, R. S., G. W. Oloo, and E. O. Amboga. 1987. An artificial diet for rearing the phytoseiid mite amblyseius-teke Pritchard and Baker. Exp. Appl. Acarol. 392: 169–174.

SAS Institute 2001. The SAS system, version 8.2 ed. SAS Institute, Cary, NC.

Tanaka, N., L. F. Steiner, K. Ohinata, and R. Okamoto. 1969. Low-cost larval rearing medium for mass production of Oriental and Mediterranean fruit flies. J. Econ. Entomol. 62: 967–968.

Taylor, D. B., J. C. Bruce, and R. Garcia. 1991. Gelled diet for screwworm (Diptera: Calliphoridae) mass production. J. Econ. Entomol. 84: 927–935.

Taylor, D. B., and R. L. Mangan. 1987. Comparison of gelled and meat diets for rearing screwworm *Cochlimyia hominivorax* (Diptera: Calliphoridae) larvae. J. Econ. Entomol. 80: 427–432.

Tsitsipis, J. A., and A. Kontos. 1983. Improved solid adult diet for the olive fruit fly, *Dacus oleae.* Entomol. Hellenicca 1: 24–29.

Vargas, R. I., H. Chang, and D. L. Williamson. 1983. Evaluation of a sugarcane bagasse larval diet for mass production of the Mediterranean fruit fly (Diptera: Tephritidae) in Hawaii. J. Econ. Entomol. 76: 1360–1362.

Urbanska, A., W. F. Tjallingii, A.F.G. Dixon, and B. Leszczynski. 1998. Phenol oxidizing enzymes in the grain aphid's saliva. Entomol. Exp. Appl. 86: 197–203.

Received 30 May 2003; accepted 5 November 2003.