COMMODITY TREATMENT

Evaluation of Mass-Rearing Procedures for *Bactrocera latifrons* (Diptera: Tephritidae)

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ABSTRACT Simple low-cost techniques and equipment have been developed for the mass production of *Bactrocera latifrons* (Hendel). A new oviposition stimulant (papaya juice) and egging cage, stackable larval rearing trays, and larval collection cabinets were developed and tested. An artificial wheat millfeed diet was modified for use as a production diet by substitution of bran for wheat millfeed. An important finding during these studies was that dilution of oviposition stimulants (fresh pepper and papaya juice) with water increased egg harvest. During 1991, these procedures were implemented and increased production of *B. latifrons* pupae by 121% over previously published methods. The application of these procedures to the development of the sterile insect technique for suppression of *B. latifrons* is discussed.

KEY WORDS *Bactrocera latifrons*, mass-rearing techniques, sterile insect technique

Bactrocera (Dacus) latifrons (Hendel) was first reported established on the island of Oahu in 1983 (Vargas & Nishida 1985b) and has since been found on the other major Hawaiian islands. Cultivated solanaceous crops such as pepper, *Capsicum annuum* L., tomato, *Lycopersicon esculentum* Miller, eggplant, *Solanum melongena* L., and feral solanaceous plants are preferred hosts (Vargas & Nishida 1985b). Demographic studies and field observations indicate that *B. latifrons* possesses a lower intrinsic rate of increase and is less of an economic pest than either the melon fly, *Bactrocera cucurbitae* (Coquillett), or the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Vargas & Nishida 1985a). Nevertheless, the presence of *B. latifrons* in Hawaii requires that all potential hosts undergo quarantine treatment before export. To provide large numbers of flies for commodity treatment studies, attractants screening, and development of sterile insect technique (SIT) procedures, we began research to develop efficient low-cost, large-scale rearing methods for *B. latifrons*.

From 1983 to 1985, *B. latifrons* was reared in the laboratory on a variety of natural and artificial diets with limited success. Since 1987, development of wheat and carrot artificial diets has allowed large-scale rearing of *B. latifrons* (Vargas & Mitchell 1987, Vargas et al. 1990). Difficulties encountered in the development of egg collection cages, large larval rearing trays, and a larval production diet have limited mass production of *B. latifrons*. In this article, we report techniques and production data for the mass-rearing of *B. latifrons*.

Materials and Methods

Studies were conducted at the Tropical Fruit and Vegetable Research Laboratory, USDA–ARS, Honolulu, HI, during 1989, 1990, and 1991. Fruit flies used to begin these studies were obtained from a colony reared for ≈50 generations as described previously by Vargas et al. (1990).

Oviposition Stimuli Tests. One liter of pupae recovered from larvae reared on a bran diet was placed inside a single cell of a double-compartment (1.22 by 1.22 by 0.61 m) metal cage similar to the cage described by Vargas (1984). Previously, we found bell pepper juice (100%) to stimulate *B. latifrons* oviposition in small yogurt cups (Vargas et al. 1990). Therefore, fresh bell pepper juice was used as the standard; papaya juice and water were the other test materials. Fresh bell pepper and papaya, *Carica papaya* L., juices were prepared by pulverizing fresh fruits in a blender and straining the mixture through a sieve.

Perforated polyethylene (10 cm long, 5 cm diameter) tubes were connected together and mounted horizontally with metal hangers inside the cage to serve as artificial oviposition sites (144 1-mm-diameter holes per tube). A cellulose sponge (2.5 by 2.5 by 7.6 cm) saturated with 20 ml of a test liquid was placed inside each oviposition tube to stimulate oviposition. In one series of experiments, we tested the effects of dilution

of pepper juice with water (1, 10, 50, 100% pure juice). In a simultaneous experiment, we compared papaya juice (one part papaya puree diluted with nine parts water) to the standard bell pepper juice (100%) and water.

Adult Density Tests. Two metal double-cell oviposition cages were stocked with one of four densities of pupae (0.5, 1, 1.5, and 2 liters); these had been reared as larvae on a bran diet. Four samples of 100 pupae were taken from each cage and held in small containers for determination of the percentage of adult emergence, sex ratio, and mortality. Adults were provided water in agar blocks and a 3:1 volumetric mixture of sugar and ICN (U.S. Biochemical, Cleveland, OH) enzymatic yeast hydrolysate. The cages were kept in a room with ceiling fluorescent lights (6.97 lux) maintained at 25 ± 1°C and 60 ± 10% RH. The photoperiod was 10:14 (L:D) h on days 1–6 and 24:00 (L:D) h on days 7–21 (ceiling fluorescent lights on at 0600 hours, day 6). Five polyethylene tubes (10 cm long, 5 cm diameter) served as artificial oviposition sites. Each oviposition tube was perforated with 144 oviposition holes (1 mm diameter, 720 holes total). Tubes without bottoms were connected together to form a single tube and hung horizontally inside the cage by metal hangers. A cellulose sponge (2.5 by 2.5 by 45.5 cm) saturated with fresh papaya juice (one part papaya puree, nine parts water) was placed inside each oviposition tube to stimulate oviposition. Sponges were cleaned and resaturated with papaya juice daily. Eggs were collected at 0800 hours on days 7–22. The volume of eggs per ml (1 ml = 15,577 ± 157 eggs [mean ± SEM], $n = 7$) obtained from females of each cage was measured with a graduated cylinder fitted with a screen to drain the water. Four samples of 100 eggs were taken daily from each cage, spread with a camel's-hair brush on moist blotting paper, and held in petri dishes for determination of hatch.

Eggs were collected at 24-h intervals for a period of 10 d from females 10–19 d old. The number of eggs laid was determined volumetrically. To determine eclosion of larvae from eggs, three samples of 100 eggs were taken daily from each tube, spread with a camel's-hair brush on moist blotting paper, and held in petri dishes for 2–3 d.

Larval Diet Tests. The larval rearing procedures using stackable plastic trays were those described previously (Tanaka et al. 1969, 1970, Vargas 1989). One hundred thousand eggs were placed on 8 liters of either a millfeed or bran diet. Table 1 summarizes the composition of larval diets used in our tests. Seven trays were stacked inside one of two larval rearing cabinets. After eggs were placed on the diet surface, tray cultures were held at 28 ± 1°C for 4 d. On day 5, tray cultures were transferred to a cooler room at 20 ± 1°C to offset larval metabolic heat and held there until the larvae matured. A photoperiod of 10:14 (L:D) h was maintained with fluorescent lights (6.97 lux). Mature larvae exited trays ad libitum and dropped into a reservoir filled with water (on days 8, 9, and 10 after egg set). For pupation, 1 liter of larvae were mixed with 6 liters of vermiculite in fiberglass boxes (45.5 by 30.5 by 15.2 cm). The following seven data sets were collected: (1) hatch of larvae from eggs, (2) duration of the developmental period from egg to pupa, (3) number of pupae recovered from eggs, (4) weight of pupae, (5) eclosion of adults from pupae, (6) number of eggs laid by adults daily over a 10-d period, and (7) hatch of larvae from F_1 eggs. Tests were replicated 10 times.

Table 1. Larval diet ingredients for *B. latifrons*

Ingredients	Wheat	Bran
Nipagen, g[a]	1.6	1.6
Granular sugar, g	20.0	20.0
Torula yeast, g[b]	35.0	35.0
Wheat (millfeed)[c]	343.4	—
Water, ml	700.0	750.0
Citric acid, g[d]	—	35.0
Carrot powder, g[e]	—	50.0
Bran[f]	—	140.0

[a] Tenneco Chemicals, Piscataway, NJ.
[b] Lake States, Rhineland, WI.
[c] Hawaiian Flour Mill, Honolulu, HI.
[d] Spectrum Chemical, Gardena, CA.
[e] General Foods, Modesto, CA.
[f] Arrowhead Mills, Hereford, TX.

Fecundity and egg-hatch determinations were based on daily egg collections from flies held for a 10-d period after a 5-d preoviposition period. Eggs were collected by inserting a small egging receptacle into a hole on the side of a 0.95-liter container (cage) of adults (Keiser et al. 1972). Adults were provided a 3:1 volumetric mixture of sugar and ICN enzymatic yeast hydrolysate and water. Eggs were spread with a small camel's-hair brush on moist blotting paper and held in petri dishes for determination of egg hatch.

Egg-Density Tests. One of three densities of eggs (100,000, 125,000, and 150,000) was placed on a bran diet and reared according to procedures in the previous experiment. Seven trays were placed in each of three separate cabinets. The following data were collected: eclosion of larvae from eggs, number of pupae recovered from eggs, weight of pupae, and eclosion of adults from pupae.

Statistical Analysis. Experiments were laid out in either a completely randomized design or a randomized complete-block design, with 4 or 10 replicates. Developmental time was calculated as a weighted mean (Seber 1973). Developmental time, pupal weight, and fecundity were transformed to natural logarithms before analyses. Percentage pupal recovery and egg hatch were subjected to an angular transformation before analysis. Data presented in tables are untransformed means. Results of the pepper juice con-

Table 2. Comparison of mean ± SEM number and percentage of total *B. latifrons* eggs collected daily from five different concentrations of pepper juice

% Concn.	% Total eggs collected	Mean no. eggs, ml/d
0	7.50 ± 1.29d	0.38 ± 0.06d
1	14.50 ± 1.29c	0.76 ± 0.05c
10	26.75 ± 2.50a	1.37 ± 0.09a
50	29.75 ± 2.99a	1.53 ± 0.21a
100	22.00 ± 1.40b	1.13 ± 0.10b

Values represent means ± SEM of four replicates. Adjacent figures in the same column followed by the same letter are not significantly different ($P > 0.05$; LSD test [SAS Institute 1985]).

centration and egg-density experiments were subjected to analysis of variance (ANOVA), and means were separated by LSD tests with type 1 error probabilities controlled by the Bonferroni method at the $P \leq 0.05$ level (Morrison 1983, SAS Institute 1985). Papaya juice data were subjected to an ANOVA, and means were separated by Tukey's studentized range (HSD) test at the $P = 0.05$ level (SAS Institute 1985). Egg-density tests were subjected to an ANOVA. Larval diet data were subjected to a t test at the $P = 0.05$ level (SAS Institute 1985).

Results

Oviposition Stimulants. Table 2 summarizes *B. latifrons* egg harvest from oviposition tubes treated with various concentrations of bell pepper juice. The highest numbers of eggs were harvested from tubes treated with 10 and 50% pepper juice, but the mean numbers harvested at these two concentrations were not significantly different. However, these concentrations did stimulate significantly greater oviposition than 0, 1, and 100% pepper juice.

Figure 1 summarizes mean egg harvest for the 10-d egg-collection period. The highest number of eggs in each of the three treatments was harvested on the second day of oviposition. Oviposition stimulants significantly (all F tests $P < 0.05$) affected numbers of eggs harvested except for day 18. The highest numbers of eggs were collected from oviposition tubes treated with papaya juice; the lowest numbers of eggs were collected from tubes treated with water. Eclosion of larvae from eggs was not affected significantly ($P > 0.05$) by treatment.

Adult Density. Egg production varied in a highly significant inverse manner per unit density of pupae (Table 3). The mean number of eggs produced in the 0.5-liter treatment was greater than the mean at 1 liter, which was in turn greater than the mean at 1.5 liters. The mean numbers of eggs produced in the 1.5- and 2-liter treatments were not significantly different.

Larval Diet. Seven variables were measured to assess bran and wheat diets for rearing *B. latifrons* (Table 4); mean values differed significantly between diets for five of the seven variables. Developmental period was significantly shorter with the bran than with the wheat diet. The bran diet yielded a significantly greater recovery of pupae, which were also significantly greater in weight than those reared on the wheat diet. The percentage adult emergence was the one variable with a significantly greater mean value with the wheat diet than with the bran diet. Percentage egg hatch and fecundity were the two variables which did not differ significantly between diets.

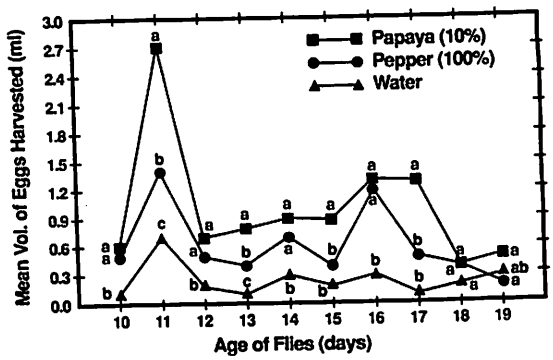

Fig. 1. Daily egg harvest from *B. latifrons* from oviposition tubes containing bell pepper juice (100%), papaya juice (10%), or water during a 10-d test period. Values represent mean of four replicates. Symbols on the same day followed by the same letter are not significantly different ($P = 0.05$; Tukey's studentized range test [HSD] [SAS Institute 1985]).

Egg-Seeding Densities. One-way ANOVA detected no significant (all F tests $P > 0.05$) variation in percentage egg hatch (mean ± SEM) (71.8 ± 3.2, 75.7 ± 2.2, 73.3 ± 5.2), percentage pupal recovery (37.1 ± 3.2, 42.2 ± 5.5, 34.1 ± 3.8), pupal weight (mg) (13.3 ± 0.3, 13.5 ± 0.3, 13.2 ± 0.3), or percentage adult emergence (84.5 ± 1.3, 84.7 ± 1.3, 83.7 ± 0.5) in diets seeded with 100,000, 125,000, or 150,000 eggs, respectively.

Mass Production of *B. latifrons* During 1990 and 1991. During 1991, mean (±SEM) monthly

Table 3. Comparison of mean ± SEM volume and percentage hatch of *B. latifrons* eggs collected daily from cages stocked with four densities of pupae

Pupal density, liter	Mean total vol. eggs, ml/15 d		% Egg hatch
	Per cage	Per liter	
0.5	44.9 ± 3.1a	89.7 ± 6.1a	76.7 ± 2.3a
1.0	66.7 ± 10.0b	66.7 ± 10.0b	76.2 ± 3.2a
1.5	48.2 ± 6.5a	32.2 ± 4.3c	75.2 ± 3.2a
2.0	72.6 ± 8.7b	36.3 ± 4.4c	78.7 ± 3.2a

Values represent means ± SEM of four replicates. Adjacent figures in the same column followed by the same letter are not significantly different ($P > 0.05$; LSD test [SAS Institute 1985]).

Table 4. Comparison of life-history parameters for *B. latifrons* larvae reared on bran and wheat diets

	Bran diet	Wheat diet	Test statistic
% Egg hatch	74.0 ± 0.6	75.4 ± 0.9	t = 1.2
Developmental time, d	8.7 ± 0.0	9.1 ± 0.0	t = 13.9*
% Pupal recovery	72.1 ± 1.9	52.6 ± 1.5	t = 8.0*
Pupal weight, mg	15.4 ± 0.1	14.5 ± 0.1	t = 6.6*
% Adult emergence	81.6 ± 1.2	87.3 ± 1.1	t = 3.6*
Fecundity (eggs/female/day)	184.7 ± 7.8	180.0 ± 7.9	t = 1.2
% Fertility	80.1 ± 2.8	74.1 ± 2.4	t = 3.6*

Values represent means ± SEM of 10 replicates. *, significant difference ($P < 0.05$; t test [SAS Institute 1985]).

production of pupae was 2,404,377 ± 166,334 pupae (Table 5) with the methods outlined in this article. This represents a >121% increase in the number of flies reared the previous year by rearing procedures described by Vargas et al. (1990).

Discussion

Vargas et al. (1990) found that fresh bell pepper juice stimulated oviposition by *B. latifrons*. In our study, we have found that dilution of bell pepper juice increased oviposition. Furthermore, harvest of *B. latifrons* eggs from oviposition tubes that contained diluted papaya juice (10%) was 66.7% higher than from tubes that contained pure bell pepper juice, the standard oviposition stimulant. Apparently, not only is a preferred oviposition stimulant important for increased deposition of eggs in bottles, but also dilution. With diluted juices, the humidity within the receptacle remains higher for a longer period of time compared with undiluted juices. Finally, papaya is grown year-round throughout Hawaii and in other tropical areas of the world where *B. latifrons* is established. Consequently, papaya juice is less expensive and should serve as a more convenient stimulant for mass production of *B. latifrons*.

Bottle egg collection cages (Vargas 1984) used to mass-rear Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), *B. dorsalis*, and *B. cucurbitae* were modified to rear *B. latifrons* by replacing large, thick polyethylene bottles with thinner polyethylene tubes. The large metal adult cage and plastic tube egging device replaced the wooden cubical cage and yogurt cup oviposition device described previously by Vargas et al. (1990).

Table 5. Mean ± SEM monthly (n = 12) production of *B. latifrons* during 1990 and 1991

	1990	1991
Eggs	3,898,892 ± 284,819	8,297,917 ± 679,005
Larvae	3,097,407 ± 199,857	6,560,207 ± 505,562
Pupae	1,087,348 ± 100,615	2,404,377 ± 166,334
% Pupal recovery	34.8 ± 1.9	37.4 ± 2.2
Pupal weight, mg	12.6 ± 0.4	13.0 ± 0.1
% Emergence	80.0 ± 1.0	80.3 ± 1.6

The inverse relationship between egg production per unit volume and volume of adults is biologically meaningful, but it need not dictate rearing strategies. The absolute quantities of eggs are probably the meaningful variable in this context, particularly because the offspring experiment detected no inverse relationships between offspring performance and numbers of eggs stocked. Thus, the four adult density treatments can be viewed as yielding 5.982 ml/liter*day × 0.5 liter = 2.99 ml eggs per day, 4.45 ml/liter* day × 1.0 liter = 4.45 ml per day, 2.144 ml/liter*day × 1.5 liter = 3.22 ml eggs per day, and 2.418 ml/liter*day × 2 liter = 4.84 ml eggs per day. These calculations suggest that rearing might be conducted with either a relatively small volume of adults (1.0 liter) to promote relatively high egg production, or at a very high adult volume (2.0 liter) to compensate for the reduced egg production per unit volume. It is noteworthy that the highest rate of egg production could not compensate for the effect of the lowest adult volume (0.5 liter); this strategy would appear to be the least appropriate for mass rearing.

During the past 5 yr, poorly understood factors associated with wheat millfeed have disrupted attempts to develop mass production techniques for *B. latifrons* on many occasions. Therefore, one practical objective of this study was investigation of a *B. latifrons* diet with a substitute bulking compound, bran. It was concluded from results of the present experiment that the bran diet was superior to the wheat millfeed diet which has been used for the last 5 yr, based primarily on the observations of greater pupal yield and size. Larval rearing trays and a metal cabinet with a water reservoir replace the small enclosed plastic trays and the wooden rearing cabinet described by Vargas et al. (1990). Pupation of larvae in vermiculite is the same procedure described previously (Vargas et al. 1987, 1990).

Methods reported here outline a low-cost mass production system for *B. latifrons*, whereby ≈500,000 insects can be produced in <100 m^2 of space in 120 h of work per week. All cages and cabinets can be constructed of sheet metal, wood, or screen. Diet ingredients are low in cost and readily available worldwide. These techniques have allowed for production of adequate numbers of flies for identification of several effective *B. latifrons* lures and development of preliminary dosimetry data for use in an SIT program. It is recognized that results of the present study were based on small tests. However, procedures developed for *B. latifrons* are comparable with those used to mass-rear *B. dorsalis*, *B. cucurbitae*, and *C. capitata* in Hawaii. Therefore, it is anticipated that millions of *B.*

latifrons can be mass reared on a routine basis. These numbers are sufficient to begin demonstration tests of the sterile insect technique for suppression of *B. latifrons*.

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