Effects of Ascorbic Acid-Rich Bell Pepper on Development of *Bactrocera latifrons* (Diptera: Tephritidae)

CHIOU LING CHANG[1] AND RICK KURASHIMA[1]

Tropical Fruit, Vegetable, and Ornamental Crop Research Laboratory, USDA–ARS, 2727 Woodlawn Drive, Honolulu, HI 96822

J. Econ. Entomol. 92(5): 1108–1112 (1999)

ABSTRACT A survey of published nutritional data of ingredients currently used in larval diet for the fruit fly *Bactrocera latifrons* (Hendel) indicated low levels of ascorbic acid in the artificial diet in comparison to a preferred host (bell pepper, *Capsicum annuum* L.). Incorporation of various amounts of green bell pepper (fresh or dehydrated powder) into larval diet significantly affected larval development by increasing pupal recovery up to 21%, adult emergence up to 17.5%, and the overall growth index. Pupal weight remained similar to that of a control diet. An optimal amount of ascorbic acid phosphate (5 mg/g diet) in the diet also improved development, especially in terms of pupal recovery and adult emergence. Addition of ascorbic acid phosphate (>15 mg/g of diet) produced detrimental effects (lengthened the larval period, decreased the pupal recovery, reduced pupal weight and overall growth index). Substituting bell pepper for carrot powder in the standard *B. latifrons* larval diet or supplementing the standard diet with ascorbic acid phosphate would be beneficial for mass rearing of *B. latifrons*.

KEY WORDS *Bactrocera latifrons*, ascorbic acid, larval diet, green bell pepper

THE FRUIT fly *Bacterocera latifrons* (Hendel) was the 4th tephritid fruit fly of economic importance to become established in Hawaii since 1983. *B. latifrons* larvae were intercepted at a southern California post office in March 1983 in a damaged package of bell peppers, *Capsicum annuum*. Subsequently, larvae were reared in Honolulu, Oahu (Anonymous 1983). *B. latifrons* has also been reported to occur in China, India, Sri Lanka, Taiwan, Malaysia, Thailand, Laos, and the Philippines (Vargas and Mitchell 1987). Bell pepper (*Capsicum annuum*, a solanaceous plant) was listed as one of the most commonly infested cultivated hosts in Hawaii (Vargas and Nishida 1985b).

Bactrocera latifrons is currently mass reared at the USDA-ARS Tropical Fruit, Vegetable & Ornamental Crop Research laboratory in Manoa (Honolulu, Oahu, HI) on a carrot powder diet developed by Vargas and Mitchell (1987). Pupal production from *B. latifrons* is currently low as compared with the other 3 species, *B. cucurbitae* (Coquillett), *B. dorsalis* (Hendel), and *Ceratitis capitata* (Wiedemann), that are being mass reared on artificial diet (Tanaka et al. 1969) in the same rearing facility.

The objective of this study was to increase pupal production by improving the larval diet. We compared the nutritional composition analysis of different vegetables with that of dehydrated carrot powder and found that although carrot powder is high in vitamin A (218,844 IU/100 g of carrot), dehydrated bell pepper contains high amounts of ascorbic acid (1,852 mg/100 g of green bell pepper and 2,051 mg/100 g of red bell pepper) (USDA-ARS 1984).

Ascorbic acid plays an essential role in the nutrition of plant feeding insects. The bollworm, *Heliothis zea* (Boddie), saltmarsh caterpillar, *Estigmene acrea* (Drury), the cotton leafworm *Prodenia litura* F., and the locust *Schistocerca gregaria* (Forsk.) failed to grow without dietary ascorbic acid (Dadd 1960, Vanderzant et al. 1962). Ito (1961) stated that ascorbic acid was a strong phagostimulant for silkworm, *Bombyx mori* (L.). Silkworm larvae reared on ascorbic acid-enriched mulberry leaves resulted in a better silk quality (Babu et al. 1992). Mahajan and Garg (1993) found that an optimal level of ascorbic acid extended the life span and decreased the reproductive potential of *Callosobruchus maculatus* (F.), the southern cow pea weevil. Singh and Sarup (1986) concluded that the optimal quantity of ascorbic acid for normal and proper development of the stalk borer *Chilo partellus* (Swinhoe) was 1.5–1.7 g/500 g diet. Kaur and Srivastava (1995) stated that 1 mg ascorbic acid per gram of diet was optimal for normal development of adult melon flies, *Bactrocera cucurbitae* (Coquillett). Based on this information, we hypothesized that ascorbic acid might play an important role in *B. latifrons* larval growth and development. Therefore, this article reports some effects of ascorbic acid-enriched larval diet on the development of *B. latifrons*. Bell pepper was selected as a host plant because of its host status availability in Hawaii and high ascorbic acid content.

Materials and Methods

Insects. *B. latifrons* eggs were collected as described by Vargas et al. 1990 from a stock colony that had been maintained at the Tropical Fruit, Vegetable and Ornamental Crop Research Laboratory for ≈200 generations. One-hour-old eggs were incubated in a nylon screened mesh bag (24°C, 65% RH) for 1 d and then placed on 50 g of larval diet.

Diets and Preparation. The composition of the standard carrot powder based-diet by weight used in this study was 5.2% carrot powder, 0.2% Nipagen, 2% sugar, 3.6% torula yeast, 2.1% citric acid, 14.5% bran, and 72.7% water (Spencer and Fujita 1997). Carrot powder is one of the key nutritional components in the diet (Vargas et al. 1990). For our tests, the following 3 types of diet were prepared: (1) the standard carrot powder diet as a control; (2) mixed diets, including (a) fresh green bell pepper plus carrot powder diet, (b) dehydrated green bell pepper powder plus carrot powder diet, (c) ascorbic acid-2-phosphate (AsA-2-P) plus carrot powder diet; and (3) substitution (replacement) diets of (a) pepper diet (carrot powder replaced by green or red pepper powder), and (b) AsA-2-P diet (carrot powder was substituted by AsA-2-P).

Fresh organically grown bell peppers were purchased from a local Market in Honolulu and homogenized in our laboratory. Dehydrated bell pepper powder (product number 6300 & 6100) was purchased from Basic Vegetable Products, Modesto, CA, and ascorbic acid phosphate (AsA-2-P or Stay C) (Sigma, St. Louis, MO) was supplied by Harvey Chan (USDA-ARS, Hilo).

Compositions of mixed diets were made by weight as follows: for fresh bell pepper diet, 100, 90, 50, and 10% of fresh bell pepper (equivalent to 50, 45, 25, and 5 g of fresh bell pepper per 50 g of mixed diet) were mixed with 0, 10, 50, and 90% of carrot powder diet (equivalent to 0, 5, 25, and 45 g of carrot powder diet per 50 g mixed diet), respectively, to make the mixed diets. For dehydrated bell pepper powder diet, 2 mixed diet combinations were chosen: (1) 2.6% carrot powder + 2.6% green pepper powder (replacing 2.6% carrot powder with 2.6% green pepper powder in standard carrot diet), and (2) 5.2% carrot powder + 5.2% green pepper (addition of 5.2% green pepper powder to the standard carrot diet). The amount of both green pepper powder and carrot powder in diet 3 was double that in diet 2.

Ascorbic acid (AsA) is an unstable substance destroyed by heat under alkaline conditions, or by exposure to oxygen in the presence of iron or copper ions. Hence, ascorbic acid-2-phosphate (Stay-C) (AsA-2-P) was used throughout this study, because it is much more stable than AsA (Koizumi et al. 1990).

AsA-2-P is a phosphate derivative of L-ascorbic acid and provides more prolonged activity of ascorbic acid in solution than does L-ascorbic acid (Saika et al. 1991). AsA-2-P also has very high stability in vitro and is easily converted to AsA in vivo by enzymatic dephosphorylation (Koizumi et al. 1990). Therefore, various concentrations of ascorbic acid phosphate (0.25, 1.25, 5, 10, 15, 20, 25 mg/g of diet) were incorporated into the carrot powder diet in this study. For replacement diets, carrot powder was replaced with different amounts of green or red bell pepper (1.3, 2.6%, 3.9, and 5.2%). The standard carrot powder diet used as control contains 5.2% carrot powder. Diets were adjusted to pH = 3.5 by using citric acid.

Bioassay. Effects of ascorbic acid-rich bell pepper on development were evaluated by the following 6 parameters: (1) larval developmental period (number of days from the day of egg hatch to pupation); (2) percent of pupal recovery (pupal production from actual eggs hatched); (3) pupal weight (milligram) (average weight per pupae); (4) growth index (percentage of pupal recovery per day of larval period); (5) percentage of adult emergence; (6) percentage of flight ability. At least 4 replicates were performed for each test.

One hundred 1-d-old eggs were placed on a strip of blotter paper in a plastic petri dish (100 by 20 mm) with 50 g of diet. Hatched larvae fed ad libitum. When larvae reached the 3rd larval stadium, before larval pop, petri dishes were placed in a 1-liter waxed cup with dry vermiculite (No. 4 grade) serving as a pupation medium. Pupae were counted, weighed, and recorded daily. The total pupae recovered from each diet was divided by the number of hatched eggs multiplied by 100% (expressed as percent pupal recovery). Daily pupal weights were totaled and divided by the total number of pupae from each diet. The duration from the day of egg hatch to the 1st d of pupation was recorded as the larval developmental period. The mean larval developmental period was calculated by using the weighted arithmetic mean (the sum of the daily pupal collections times the number of days to pupation divided by total number of pupae pupated) (Sanders 1990). Percentage of adult emergence and flight ability tests were modifications of those devised by Boller et al. (1981). A black Plexiglas tube (8.25 cm i.d., 20 cm high) was fitted into a black painted petri dish (9 cm diameter) that was lined with 1 cm wide paper strips. The insides of tubes were lightly coated with Talcum powder to prevent the flies from climbing out (tubes were tapped on a firm surface to remove excess talc, and the talc was wiped off of the bottom 1 cm of the tube to provide resting places for newly emerged flies). Two days before adult emergence, pupae were placed in the petri dish. After emergence, insects remaining in the dish were categorized as follows: adult unemerged, partially emerged, emerged but malformed (including nonfliers and unexpanded wings), and normal. Percentage of emergence was calculated as the total number of pupae minus the number of unemerged and partially emerged flies divided by total pupae times 100%. Percentage of fliers was derived from the total number of pupae minus the number of unemerged, partially emerged, emerged but malformed divided by total number of emerged flies multiplies by 100%. The growth index was calculated as percentage of pupal recovery divided by the

Table 1. Effect of bell pepper (fresh and dehydrated) on development of *B. latifrons*

Pepper + carrot diet	Larval period, days	Pupal recovery, %	Pupal wt, mg	Growth index, %/day	% adult emergence	Fliers, %
Fresh green bell pepper						
100% carrot diet (control)	9.31 ± 0.07c	77.43 ± 0.01b	15.24 ± 0.21a	8.32 ± 0.09b	62.75 ± 2.02b	—
10% pepper + 90% carrot diet	9.22 ± 0.09c	83.82 ± 0.01ab	15.21 ± 0.20a	8.94 ± 0.15ab	69.75 ± 1.49ab	—
50% pepper + 50% carrot diet	9.05 ± 0.19c	92.17 ± 0.02a	14.49 ± 0.18a	10.21 ± 0.41a	75.25 ± 2.02a	—
90% pepper + 10% carrot diet	13.64 ± 0.36b	41.62 ± 0.03c	8.68 ± 0.17b	3.07 ± 0.31c	27.50 ± 3.57b	—
100% pepper diet	17.25 ± 1.49a	38.82 ± 0.08c	6.46 ± 0.58c	2.40 ± 0.61c	24.25 ± 7.17b	—
F	27.89	38.00	172.95	83.06	39.29	—
df	15	15	15	15	15	—
P > *F*	0.0001	0.0001	0.0001	0.0001	0.0001	—
Dehydrated bell pepper powder						
5.2% carrot powder (control)	8.32 ± 0.04b	68.28 ± 0.04b	15.90 ± 0.50b	8.20 ± 0.51b	54.00 ± 3.34b	32.31 ± 2.11a
2.6% green pepper + 2.6% carrot powder	7.82 ± 0.08c	86.61 ± 0.75a	17.48 ± 0.23a	11.09 ± 0.15a	69.50 ± 1.04a	42.45 ± 9.13a
5.2% green pepper + 5.2% carrot powder	8.22 ± 0.07b	89.27 ± 0.50a	16.53 ± 0.21ab	10.86 ± 0.11a	71.50 ± 1.55a	48.19 ± 3.49a
5.2% green pepper + 0% carrot powder	8.14 ± 0.08b	88.88 ± 0.83a	16.90 ± 0.25ab	10.92 ± 0.18a	69.75 ± 1.65a	45.81 ± 3.07a
5.2% red pepper + 0% carrot powder	8.01 ± 0.07bc	90.97 ± 0.74a	15.90 ± 0.06b	9.42 ± 0.20a	69.75 ± 3.42a	37.78 ± 1.80a
F	74.33	533.48	7.95	213.49	5.89	1.80
df	15	15	15	15	15	15
P > *F*	0.0001	0.0001	0.0012	0.0001	0.0047	0.1814

Within a column means followed by different letters were statistically different ($\alpha = 0.05$, ANOVA test).

larval developmental period in days as adopted by Srivastava et al. (1978).

Statistical Analysis. Data are reported as means ± SE. Differences among treatment means were determined by analysis of variance (ANOVA), with the honestly significant difference (HSD) value calculated as a Tukey statistic at $P = 0.05$ (Snedecor and Cochran 1967), using SAS system computer software (SAS Institute 1987).

Results and Discussion

Effects of Fresh Bell Pepper on the Development of *B. latifrons*. The addition of fresh green bell pepper (50%) to the carrot powder diet caused a significant increase in percentage of pupal recovery (15%), emergence (13%), and growth index (2%), whereas pupal weight remained similar to the control (Table 1). Ninety and 100% green-bell-pepper-enriched diet prolonged the developmental periods, reduced the pupal recovery, decreased the pupal weight, and significantly lowered growth indices and adult emergence. This substandard performance could be caused by either too much ascorbic acid or mold formation on the diet, or both.

Effects of Bell Pepper Powder on the Development of *B. latifrons*. Dehydrated pepper powder and carrot powder mixed diets consisting of 2.6% carrot powder + 2.6% green pepper powder and 5.2% carrot powder + 5.2% green pepper powder affected pupal recovery, growth indicies, and adult emergence (Table 1). The larval development period in 2.6% pepper + 2.6% carrot mixed diet was significantly shorter than larval period for the carrot powder diet. Bell pepper may contain a feeding stimulant factor or nutritional component that caused the larvae to develop faster. Pupal recovery from both the mixed diets above were significantly higher than those from the control carrot powder diet by 18 and 21%, respectively (Table 1). Pupal recovery of replacement diets were either the same as the control (1.3, 2.6, 3.9% of green pepper powder) or significantly increased by 20 or 22% from 5.2% of green or red pepper powder, respectively (Tables 1 and 3).

Pupae from the mixed diet containing 2.6% pepper + 2.6% carrot powder were significantly heavier than pupae obtained from the control diet, whereas pupal weights of other treatments remained the same as the control. Overall, growth indices for insects reared in carrot powder-bell pepper mixed diets and the 5.2% green or red pepper replacement diets were significantly higher than growth indices from control diets by 2.8, 2.4, 2.7, and 1.2% per developmental day (Table 1). Adult emergence was also higher for insects reared on the above mentioned mixed and replacement diets than for those on control diets. Flight ability was similar for adults reared from both the carrot powder diet and diets containing bell pepper (Tables 1).

Effects of Ascorbic Acid Phosphate on Development of *B. latifrons*. We have shown that addition of bell pepper to the diet of *B. latifrons* promotes larval growth and development (Table 1), especially in pupal recovery, percentage of adult emergence, and growth index. Further tests with diets containing ascorbic acid −2–phosphate were initiated based on the assumption that high ascorbic acid levels in bell

Table 2. Effect of exogenous AsA-2-P to carrot diet on development of *B. latifrons*

Concn, mg/g diet	Pupal recovery, %		Growth index, %/day		% adult emergence	
	A	B	A	B	A	B
0 (Control)	28.47 ± 1.28d	74.79 ± 2.26b	2.72 ± 0.14de	7.63 ± 0.27cd	56.00 ± 5.40c	81.05 ± 3.39bc
0.25	33.09 ± 3.77cd	79.18 ± 2.78b	3.20 ± 0.35cd	8.56 ± 0.35bc	77.68 ± 0.50ab	96.85 ± 1.44ab
1.25	41.28 ± 1.60c	85.03 ± 2.24ab	4.18 ± 0.19c	9.41 ± 0.30ab	88.10 ± 2.85a	97.56 ± 1.59a
5.00	88.48 ± 3.07a	90.71 ± 0.37a	9.18 ± 0.27a	9.80 ± 0.04a	85.62 ± 2.28a	74.55 ± 5.11cd
10.00	73.91 ± 1.34b	81.29 ± 0.78ab	7.27 ± 0.10b	8.34 ± 0.08c	62.95 ± 5.90bc	63.00 ± 3.88d
15.00	76.61 ± 2.55ab	75.61 ± 2.33b	6.42 ± 0.21b	6.77 ± 0.22d	54.94 ± 1.63c	36.41 ± 2.49c
20.00	30.96 ± 4.17cd	46.59 ± 1.75c	2.03 ± 0.27e	3.33 ± 0.11e	52.19 ± 5.64c	61.47 ± 4.50d
25.00	2.04 ± 0.86e	13.91 ± 3.57d	0.11 ± 0.04f	0.82 ± 0.21f	66.67 ± 23.57c	53.99 ± 6.01d
F	130.82	132.00	192.95	197.34	10.65	32.91
df	24	24	24	24	24	24
P > *F*	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Within a column means followed by different letters were statistically different (α = 0.05, ANOVA test). A and B represent both low and high levels of pupal recovery from control diets.

pepper might be the triggers for increasing pupal recovery of *B. latifrons*.

Pupal recoveries vary with changes in rearing conditions and both mass and small-scale fruit fly rearings are affected by a variety of known and unknown influences. Therefore, wide fluctuations of pupal recoveries sometimes are observed over a course of time. In this article, we report the effect of ascorbic acid phosphate on pupal recovery, percentage of adult emergence and growth index from 2 sets of data representing 2 different levels of pupal recoveries in control. Both 28.27 ± 1.28% and 74.79 ± 2.26% in Table 2 reflect pupal recoveries at low (Table 2, A) and high (Table 2, B) levels, respectively.

An ascorbic acid 2-phosphate concentration of 5 mg/g diet in carrot powder diet showed significant promise over both lower and higher concentrations of AsA-2-P, regardless of level of pupal recovery (Table 2). Where pupal recovery was low (Table 2), larvae benefited from concentrations of AsA-2-P between 1.25 and 15 mg/g, showing significantly increased pupal recovery over the control diet. These increases were 12.82, 60.00, 45.44, and 48.15% for AsA-2-P concentrations of 1.25, 5.0, 10.0, and 15 mg/g, respectively (Table 2). However, AsA-2-P concentrations <1.25 or higher than 15 mg/g resulted in poor pupal recovery (Table 2). Larvae seemed to benefit from AsA-2-P where pupal recovery was high (Table 2), although such benefits were not as striking as when pupal recovery was low (Table 2). Here, concentrations of AsA-2-P <15 mg/g did not significantly increase in pupal recovery, except at the 5 mg/g AsA-2-P level (Table 2). This may be explained by addition of ascorbic acid promoting enzymatic hydroxylations, thus increasing survivorship (Rockstein 1978).

Similar results were shown with other parameters such as percentage of adult emergence and growth index. Percentage of emergence and growth indices were the highest at 5 mg/g AsA-2-P among the tested concentrations of AsA-2-P with low pupal recovery (A). At high pupal recovery (B), AsA-2-P concentrations between 1.25 and 5 mg/g are needed in the diet to achieve the significant benefit over the control diet (Tables 2 and 3). Larval developmental period, pupal weight, and flight ability were not significantly affected by AsA-2-P.

The purpose of this study was to evaluate the addition of fresh or powdered bell pepper and ascorbic acid and their effects on larval development and adult fitness of mass-reared *B. latifrons*. The nutritional composition of bell pepper led us to suspect that ascorbic acid could provide an important supplement to the standard *B. latifrons* diet. In our tests, addition of bell pepper or ascorbic acid at certain concentrations promoted pupal production and increased adult emergence. These effects were more apparent where pupal recovery levels were low. Substituting 1.3% green pepper powder for carrot powder in the diet or the ad-

Table 3. Effect of green pepper powder replacement diet on development of *B. latifrons*

	Pupal recovery, %		Growth index, %/day		% adult emergence	
	A	B	A	B	A	B
0.0% Pepper	28.47 ± 1.28b	74.79 ± 2.26a	2.72 ± 0.14b	7.63 ± 0.27b	56.00 ± 5.40b	81.05 ± 3.39b
1.3% Pepper	26.67 ± 6.33b	78.64 ± 0.03a	2.49 ± 0.54b	9.03 ± 0.37ab	71.63 ± 7.72ab	92.84 ± 0.50a
2.6% Pepper	27.91 ± 4.62b	72.27 ± 0.03a	2.74 ± 0.42b	8.14 ± 0.30a	83.07 ± 1.36a	94.03 ± 1.94a
3.9% Pepper	31.64 ± 1.83b	81.41 ± 0.04a	3.17 ± 0.15b	9.14 ± 0.44a	80.27 ± 2.33a	94.30 ± 0.95a
5.2% Pepper	51.95 ± 3.64a	84.88 ± 0.03a	5.49 ± 0.42a	9.47 ± 0.25a	80.32 ± 4.51a	64.55 ± 2.33c
AsAP (5 mg)	—	85.49 ± 4.96a	—	8.60 ± 0.64a	—	94.47 ± 2.02a
F	7.01	2.80	11.19	5.19	5.27	37.92
df	15	19	15	19	15	19
P > *F*	0.0022	0.0642	0.0002	0.0079	0.0074	0.0001

Within a column means followed by different letters were statistically different (α = 0.05, ANOVA test). A and B represent both low and high levels of pupal recovery from control diets.

Table 4. Cost of carrot powder substitutes for mass rearing of *B. latifrons*

Carrot powder substitutes	Cost ($) per tray
5.2% carrot powder (control)	1.30
AsA-2-P (5 mg/g diet) + carrot powder (5.2%)	1.57
AsA-2-P (5 mg/g diet)	1.08
1.3% pepper powder	0.91
2.6% pepper powder	1.82
3.9% pepper powder	2.73
5.2% pepper powder	3.64

dition of ascorbic acid phosphate (5 mg/g diet) to either the carrot diet or a diet without carrot powder may be a cost-effective alternative to the carrot-based diet (currently used for mass production of *B. latifrons*). The cost per tray for the addition of these ingredients was $ 0.91 per tray for 1.3% pepper substitution or $1.08 per tray for addition of 5 mg AsA-2-P per gram of diet (Table 4).

This study not only demonstrated the effects of ascorbic acid-rich bell pepper on development of *B. latifrons*, but also provided us with a basis for further nutritional studies of fruit flies.

Acknowledgments

We thank Chris Albrecht and Cherilyn Wilhelm for their hard work in collecting and analyzing the data. We also thank John Spencer (USDA-ARS, Honolulu) for numerous insect colony supplies from his group. Appreciation also goes to Harvey Chan (USDA-ARS, Hilo) for supplying the ascorbic acid phosphate and critical review of the manuscript. Ernest Harris (USDA-ARS, Honolulu) and David Lance (USDA-APHIS-PPQ-HPPC, Waimanalo, HI) for their critical reviews of an early version of the manuscript, and Eric Jang (USDA-ARS, Hilo) and Don McInnis (USDA-ARS, Honolulu) for their critical editorial reviews of final version of the manuscript.

References Cited

Anonymous. 1983. Hawaii Department of Agriculture. Dep. Off. Agric. Pest Rep. 3(1–4): January–December.

Babu, M., M. T. Swamy, P. Kameswara Rao, and M. Seshagiri Rao. 1992. Effect of ascorbic acid-enriched mulberry leaves on rearing of *Bombyx mori* L. India J. Seric. 31(2): 111–114.

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

Dadd, R. H. 1960. Some effects of dietary ascorbic acid on locusts. Proc. R. Soc. 53: 128–143.

Ito, T. 1961. Effect of dietary ascorbic acid on the silkworm, *Bombyx Mori.* Nature (Lond.) 192: 951–952.

Kaur, S., and B. G. Srivastava. 1995. Effect of ascorbic acid and its different quantities on various parameters of reproductive potential of *Dacus cucurbitae* (Coquillett). Indian J. Entomol. 57: 151–156.

Koizumi, S., A. Maruyama, and T. Fujio. 1990. Purification and characterization of ascorbic acid phosphorylating enzyme from *Pseudomonas azotocolligans.* Agric. Biol. Chem. 54: 3235–3239.

Mahajan, S., and S. K. Garg. 1993. Effect of ascorbic acid on longevity and reproductive characteristics of *Callosobruchus maculatus* F. Bionature 13: 21–23.

Rockstein, M. 1978. Biochemistry of insects. Academic, New York.

Saika, S., R. Kanagawa, K. Uenoyama, K. Hiroi, and J. I. Hiraoka. 1991. L-ascorbic acid 2-phosphate, a phosphate derivative of L-ascorbic acid, enhances the growth of cultured rabbit keratocytes. Graefe's Arch. Clin. Exp. Ophthalmol. 229: 79–83.

Sanders, D. H. 1990. Statistics—a fresh approach. McGraw-Hill, New York.

SAS Institute. 1987. SAS user's guide: statistics, version 6.12 ed. SAS Institute Inc.

Singh, R., and P. Sarup. 1986. Significance and optimal quantity of ascorbic acid in artificial diets for the mass rearing of maize stalk borer, *Chilo partellus* (Swinhoe) (Lepitoptera: Crambidae). J. Entomol. Res. 10: 174–179.

Snedecor, G., and W. Cochran, W. G. 1967. Statistical methods, 6th ed. The Iowa State University Press, Ames.

Spencer, J. P., and B. H. Fujita. 1997. A procedural manual for mass rearing four species of Tephritid fruit flies. USDA-ARS, PWA, USPBARC rearing facility at 2727 Woodlawn Drive, Honolulu, Hawaii.

Srivastava, B. G., N. C. Pant, and H. S. Chaudhry. 1978. Effect of ascorbic acid on *Dacus cucurbitae* (Coquillett) maggots in the artificial diet under aseptic condition. Indian J. Entomol. 40: 384–387.

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. Econo. Entomol. 62: 967–968.

Vanderzant, E. S., M. C. Pool, and C. D. Richardson. 1962. The role of ascorbic acid in the nutrition of three cotton insects. J. Insect Physiol. 8: 287–297.

Vargas, R. I., and S. Mitchell. 1987. Two artificial larval diets for rearing *Dacus latifrons* (Diptera: Tephritidae). J. Econ. Entomol. 80: 1337–1339.

Vargas, R. I., and T. Nishida. 1985b. Survey for *Dacus latifrons* (Diptera: Tephritidae0. J. Econ. Entomol. 78: 1311–1314.

Vargas, R. I., S. Mitchell, B. Fujita, and C. Albrecht. 1990. Rearing techniques for *Dacus latifrons* (Hendel) (Diptera: Tephritidae). Proc. Hawaii. Entomol. Soc. 30: 71–78.

[USDA-ARS] U.S. Department of Agriculture, Agricultural Research Service. 1984. Composition of foods: vegetables and vegetable products; raw, processed, prepared. Agricultural handbook no. 8–11. Consumer and Food Economics Resarch Division, Agricultural Research Service, USDA, Beltsville, MD.

Received for publication 21 October 1998; accepted 27 May 1999.