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# Effect of Amino Acids on Larvae and Adults of *Ceratitis capitata* (Diptera: Tephritidae)

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**ABSTRACT** Grouped or individual amino acids from two diets were deleted to evaluate the effects of amino acids on larvae and adults of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Larvae died when fed with diets free of 10 exogenous essential amino acids (arginine, isoleucine, leucine, lysine, histidine, methionine, phenylalanine, threonine, tryptophan, and valine) or containing nine exogenous amino acids with removal of any one of the 10 essential amino acids. However, when larvae were reared on diets lacking all eight of the exogenous nonessential amino acids together (alanine, aspartic acid, cystine, glutamic acid, glycine, proline, serine, and tyrosine), or either glycine or serine, they survived but exhibited significantly delayed larval development. When adults fed on a diet lacking all 10 essential amino acids or all eight nonessential amino acids, no effect on adult survivorship, sexual maturity, or egg hatch was observed, but the fecundity was significantly reduced. Removal of arginine, histidine, isoleucine, leucine, lysine, threonine, tryptophan, methionine, tryptophan, or valine from adult diets decreased fecundity significantly.

**KEY WORDS** amino acids, glycine, serine, larval diet, insect diet

THE NUTRITIONAL VALUE OF PROTEIN depends on its amino acid contents. The digestive system of animals hydrolyzes ingested proteins into amino acids and amino acids then were combined into the specific proteins needed for growth and development. Besides the roles of amino acids in protein synthesis, high levels of free amino acids have additional functions related to neural transmission, detoxification, and synthesis of phospholipids, energy production, and morphogenetic processes that have important biological roles (Chen 1985).

Several researchers have reported the effects of amino acids on insect development are dose- and species-dependent (Hinton et al. 1951; Friend et al. 1957; Tsiropoulos 1977, 1978, 1980, 1983; Ferro and Zucoloto 1990; Anand and Anand 1994; Kaur and Srivastava 1994; Cangussu and Zucoloto 1997; Zografou et al. 1998; Chang et al. 2001). Hinton et al. (1951) found that a syndrome of phenotypic abnormalities occurred when *Drosophila melanogaster* Meigen were reared with a high concentration of L-tryptophane, isoleucine or serine, and glycine.

The first successful experiment to establish nutritionally essential and nonessential amino acids was carried out with young rats by Rose (1938). Rose defined an essential amino acid (EAA) as one that when omitted from the diet caused subnormal or no growth, and a nonessential amino acid (NEAA) as one that when deleted does not affect normal growth. By deleting one amino acid at a time from the diet, Rose (1938) established that arginine, isoleucine, leucine,

lysine, histidine, methionine, phenylalanine, threonine, tryptophan, and valine were EAAs for rats. Using Rose's deletion method, the amino acid requirements for some 20 insects have been determined. The 10 EAAs required by the rat are essential for the majority of the insects studied. *Delia antique* (Meigen), *Pectinophora gossypiella* (Saunders), and *Trogoderma granarium* Everts are examples of insects with identical requirements (House 1962). Several amino acids that are nonessential for the rat have been shown to be essential for some insects. Proline is essential for *Phormia regina* Meigen; proline and serine for male *Blattella germanica* (L.) (House 1962). Cystine is essential for *Aedes aegypti* (L.) (Gilmour 1961). Glycine is a growth-promoting factor for *D. melanogaster* (Hinton et al. 1951), *Calliphora* spp. (Gilmour 1961), *Podocarpus affinis* Sprengel (House 1954), *Oryzaephilus surinamensis* (L.) (Davis 1956), and perhaps essential for the mosquito *A. aegypti* (Golberg and DeMeillon 1948, Singh and Brown 1957).

Although the importance of protein is well defined and recognized in some insects and other animals, the association between amino acids and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is poorly understood. Studies on the amino acid requirements in fruit fly rearing especially in tephritids by using the deletion method have been hindered by the difficulty in preparing chemically defined diets. Chang et al. (2001b) recently developed a complete chemically defined diet for *C. capitata* adult (*C. capitata* 2 diet) and a meridic diet (only one ingredient with

**Table 1.** Composition of *C. capitata* 1 larval diet and 2 adult diet

Constituents	<i>C. capitata</i> 1 (mg/50 g diet)	<i>C. capitata</i> 2 (mg/50 g diet)
L-arginine	106.80	106.80
L-histidine	45.60	45.60
L-isoleucine	56.40	56.40
L-leucine	108.00	108.00
L-lysine	58.80	58.80
L-methionine	27.60	27.60
L-phenylalanine	70.80	70.80
L-threonine	54.00	54.00
L-tryptophan	28.80	28.80
L-valine	79.20	79.20
L-alanine	78.00	78.00
L-aspartic acid	112.80	112.80
L-cysteine	40.80	40.80
L-glutamic acid	392.40	392.40
L-glycine	90.00	90.00
L-proline	124.80	124.80
L-serine	78.00	78.00
L-tyrosine	48.00	48.00
Ribonucleic acid	100.00	100.00
Sugar	2000.00	8000.00
Cholesterol	40.00	80.00
Vitamin mixture <sup>a</sup>	36.35	424.85
Salt mixture no. 185 (McCullum & Davis)	100.00	100.00
Streptomycin	50.00	50.00
Tetracycline 343	5.00	5.00
Methylparaben	100.00	100.00
Sodium benzoate	100.00	100.00
Citric acid	500.00	500.00
Agar	—	400.00
Corncob	12000.00	—
Water	33000.00	40000.00
Total	49662.15	51086.01

<sup>a</sup> In Chang et al. (2001).

unknown chemical structure) for larvae (*C. capitata* 1 diet) for fruit fly nutritional studies. This is an ascertained report on the effects of omitting amino acids from the above-mentioned adult and larval diet of the Mediterranean fruit fly. Eighteen common amino acids that normally make up proteins in feeds were evaluated in this study. Ten of these are essential because they can be only obtained from the diet and the other eight are not essential because they are synthesized in the body from other amino acids.

### Materials and Methods

**Insects.** Eggs from 1-h collection and 2-d-old pupae of *C. capitata* were obtained from colonies of the Maui Med-93 strain maintained at the rearing unit of USDA-ARS, Pacific Basin Agricultural Research Center in Honolulu, HI. This colony has been reared for ≈700 generations on a modified diet of Tanaka et al. (1969). Within 6 h after emergence, larvae and adults were transferred into designated containers with different test diets and used as the starting stages, respectively, for bioassay throughout this study.

**Diet Preparation.** Diets used in this study were meridic larval diet (*C. capitata* 1) and holidic adult diet (*C. capitata* 2) (Chang et al. 2001a,b) (Table 1). The required amounts of the 10 EAAs (arginine, isoleu-

cine, leucine, lysine, histidine, methionine, phenylalanine, threonine, tryptophan, and valine) and the eight NEAAs (alanine, aspartic acid, cystine, glutamic acid, glycine, proline, serine, and tyrosine) (Table 1) were prepared as described by Chang et al. (2001a,b).

In general, larval diets were prepared as follows. A mixture of 18 amino acids and the vitamin mixture were each prepared in bulk (Table 1). They were combined with other ingredients: sugar (McCullum & Davis, Aurora, OH), salt mixture no. 185, cholesterol, methylparaben, sodium benzoate, streptomycin, oxytetracycline HCl, citric acid, and ribonucleic acid. All dry materials (including 12 g of corncob bulking agent) were weighed into sterile polyethylene Stomacher blender bags (80 ml, 16 by 10 cm) and mixed in a Stomacher laboratory blender (400-ml capacity) (Daigger and Company, Lincolnshire, IL) at the normal-speed setting for 60 s. Tap water (55°C, 33 ml) was then added into this diet mixture and mixed in the Stomacher blender for an additional 120 s at high speed. Bags with diet mixtures were labeled and stored in a refrigerator (4°C) for later use. Before use, diets were taken from refrigeration to a 24 ± 1°C, 65 ± 1% RH room and mixed at normal-speed setting for 60 s.

The composition of the *C. capitata* 2 adult diet is shown in Table 1. All chemicals listed were purchased from ICN Pharmaceuticals, Inc. (Costa Mesa, CA), weighed, mixed, and heated in a 100-ml beaker on a hot-plate (55°C) with a constant mixing (Daigger and Company, Inc., Vernon Hills, IL). Agar was added and heated until the solution became clear. The agar mixture was poured into petri dishes, and agar diets were stored in the refrigerator (4°C) for later use.

In this study, exogenous EAAs or NEAAs were deleted in tally or individually from both *C. capitata* 1 larval diet and *C. capitata* 2 adult diet, respectively, to determine the effects of amino acids to *C. capitata*. The protein hydrolysate:sugar diet (1:3) and Tanaka's mill-fed diet (Tanaka et al. 1969) were used as the standard diets for adult or larvae, respectively.

**Bioassays.** The importance of amino acids in *C. capitata* was determined by evaluation of developmental and reproductive parameters as described by Chang et al. 2001: larval developmental period (days), pupal recovery (percentage), pupal weight (milligrams), adult emergence (percentage), flier (whether adult flies can fly) (percentage), sexual maturity, fecundity (number of eggs) and egg hatch (percentage) by using the above-mentioned deletion method. All treatments and tests were repeated at least four times.

**Effect of EAAs on Larval Development.** Fifty newly eclosed larvae were randomly selected from 1 ml of egg collection and were transferred onto a strip of blotting paper on top of 50 g of diet inside a sterile polyethylene bag by using a fine brush. Four bags of each test diet were prepared individually. Each bag was stapled on the creases of the bag and maintained at 24 ± 1°C and 65 ± 1% RH. When larvae reached third instar, polyethylene bags were opened and placed in a 1-liter waxed cup with vermiculite for pupation. Pupae were counted and weighed as soon as brown puparia were formed. Pupae recovered from

each diet were expressed as percentage of recovery of neonate larvae used. Daily pupal weights were totaled and divided by the total number of pupae from each diet to calculate mean pupal weight. The larval development period was measured from egg hatch to the first day of pupation. The mean larval developmental period was calculated by using a weighed arithmetic mean – the sum of the daily pupal collections times the number of days to pupation divided by total number of pupae. Adult emergence and flight ability were determined according to Chang et al. (2001). Percentage of fliers was derived from the total number of pupae minus the number of unemerged, partially emerged, emerged but deformed, and nonfliers divided by the total number of emerged flies and multiplied by 100. All emerged adults from each test were combined and placed in a metal cage (26.5 by 26.5 by 26.5 cm) for egg collection. Eggs were collected when adults were 6 d old at 25°C and continued for four consecutive days. Four hundred eggs per diet per day were transferred into four strips of blotting papers (100 eggs each) inside a petri dish for egg hatch. Percentage of egg hatch was calculated as mean number of larvae eclosed from each 100 eggs times 100. A deletion technique was used to determine the importance of nutrients in *C. capitata* 1 diet.

Groups and individuals of either essential amino acids or nonessential amino acids were systematically deleted from *C. capitata* 1 meridic diet, and the effects of amino acids in the diet were evaluated.

**Effect of Amino Acids on Adults.** Grouped and individual amino acids either essential or nonessential amino acids were deleted from the chemically defined (*C. capitata* 2) diet to determine their importance in the diet. The protein hydrolysate:sugar diet (1:3) was again the standard diet.

Small- and large-scale tests were conducted. In a small-scale test, in general, at 5 d posteclosion at 25°C, 10 mating pairs of *C. capitata* from each treatment were transferred to a 1-liter waxed cup with test diet provided in a vial cap (1 cm in diameter) at the bottom. Eggs from test diet-fed females were collected and counted every other day starting from 6 d postemergence for 18 d (nine collections) from both eggging devices and food (eggs often were laid into the agar food provided). The eggging device was made by inserting a bottomless sauce cup into another with a piece of 25-pinholed paraffin between. Dead flies from each waxed cup were recorded by sex every other day and discarded. Percentage of survivorship was calculated as total flies (10 each sex) minus dead flies divided by 10 times 100. The number of eggs per female per day (fecundity) was calculated as the 18 d cumulative eggs counted divided by the mean number of females surviving and the number of collecting days. Four collections from each test diet were performed. Four hundred eggs collected each day from 6-, 7-, 8-, or 9-d-old females fed on test diets were used to determine fertility (egg hatch). Newly emerged *C. capitata* adults were sexed and placed in metal cages in a separate room with identical environmental conditions (25°C, 65% RH, and a photoperiod of 12:12

[L:D] h) and supplied with test diets. To test flight ability, 25 flies of each sex, from each diet, were collected daily (on days 1–6) and placed in 1-liter wax cups. Flies were fast chilled in a –10°C freezer. As soon as they were immobile ( $\approx 1$  min), the flies were removed from the freezer and flight ability was tested as described by Chang et al. (2001). After 4 d, flies remaining in the dishes were counted as nonfliers and the numbers were expressed as a percentage of the total. Percentage of fliers was derived from 100% minus percentage of nonfliers.

Large-scale tests were conducted to ensure our results from the small-scale tests. In large-scale tests, postoviposition (laid eggs) eggs were collected daily from adults emerging from 20 g of pupae ( $\approx 2,000$  pupae, sex ratio is  $\approx 1:1$ ). Daily collected eggs were measured volumetrically and data presented as proportional to those from flies fed with original *C. capitata* 2 diet. Eggs were collected from 3-d-old females for 2 wk. Data of egg counts from each treatment were presented as proportional to those from control.

**Sexual Maturity.** The number of preoviposition eggs (mature eggs in ovaries) accounted for sexual maturity for flies fed with different diets. Adults of *C. capitata* were held in a metal-screened cages (26.5 by 26.5 by 26.5 cm) and fed with 18 amino acids, 10 EAAs, eight NEAAs, or threonine-free diets. Twenty females from each treatment were fixed in 70% alcohol and glycerin (1:1) solution for at least 48 h, and ovaries were dissected daily from 3 to 16 d of age. The total number of mature oocytes in each ovary was counted and recorded. The appearance of the mature oocytes in the ovaries determined the degree of sexual maturity.

**Fertility.** Four hundred eggs per treatment from the first 4 d during oviposition (sixth–ninth) were carried out for an egg hatchability test.

**Statistical Analysis.** Data are summarized using PROC Univariate and presented as mean  $\pm$  SE. Differences among means were determined by multiple *t*-test in analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) (SAS Institute 2002).

## Results and Discussion

**Effect of EAAs on Larval Development.** No larvae were recovered from the diet lacking in exogenous EAAs totally or singly. Figure 1 shows the maximal body length of larvae before death at 18 d old. Survival was zero for larvae reared on a diet without exogenous threonine or tryptophan during the 18-d period. These results indicate the importance of EAAs to *C. capitata* larvae, especially threonine and tryptophan. Friend et al. (1957) found that all test larvae of the maggot *D. antique* died in the first instar when were reared on diets lacking one of the following amino acids: L-arginine, L-histidine, L-isoleucine, L-tryptophan, or L-valine. Friend et al. (1977) also found that larvae feeding on diets lacking either L-phenylalanine or L-threonine died before the third instar, and those fed diets lacking L-leucine, L-lysine, or L-methionine died

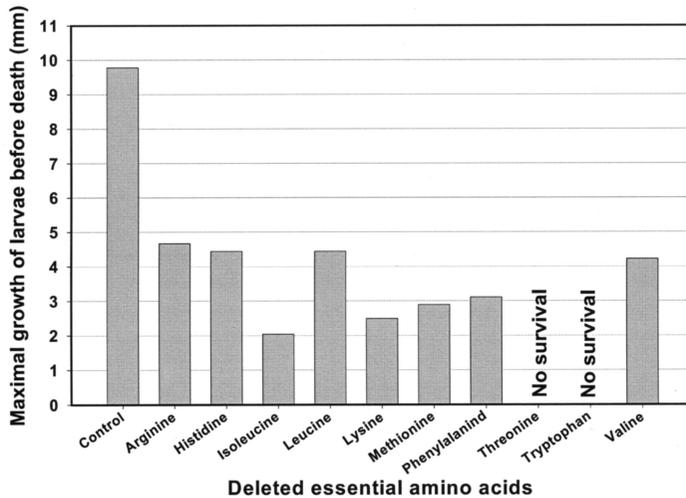


Fig. 1. Larvae of *C. capitata* reared on the meridic larval diet *C. capitata* 1 diet without exogenous EAAs for 18 d resulted in zero pupal recovery. The bars represent the length of maximal larval growth (millimeters) before death.

before reaching the pupal stage. Our results are consistent with their findings that the 10 essential amino acids are indispensable to tephritid fruit flies.

**Effect of NEAAs on Larval Development.** *C. capitata* larvae fed with diet lacking all eight exogenous NEAAs in group delayed larval development where omitting serine singly extended larval development and surplus weight loss (Table 2). In addition to these two effects, larvae reared on diet without glycine decreased adult emergence as well (Table 2). Cangussu and Zucoloto (1997) reported that protein deficiency during the immature phase reduced adult emergence, adult female size, and oocyte maturation and increased duration of the larval phase of *C. capitata*. The results of the present work are consistent with the findings of Cangussu and Zucoloto (1997). Larvae reared on a diet without exogenous alanine, aspartic acid, cystine, glutamic acid, proline, or tyrosine, respectively, did not show any significant difference in all the test parameters compared with the complete meridic diet (*C. capitata* 1) (Table 2). Similar observation was found by Friend et al. (1957) by

using diets that lacked L-alanine, L-aspartic acid, L-cysteine, L-glutamic acid, glycine, L-hydroxyproline, L-proline, L-serine, or L-tyrosine to rear larvae of *H. antique*. The discrepancies with glycine and serine between Friend et al. (1957) and present findings could be the difference in dosages. According to Hinton et al. (1951), high concentrations of glycine and serine are extremely toxic and have an inhibitory effect on the growth of *D. melanogaster*. However, high levels of ribonucleic acid can act as an antagonist to this inhibition. The interaction between these substances requires further studies.

**Effect of Amino Acids on Adults.** Pre- or postoviposition egg productions was significantly reduced ( $P < 0.0001$ ) for adults of *C. capitata* fed with the holidic (*C. capitata* 2) diet excluding all the amino acids, the 10 EAAs, or one of the 10 EAAs, or the eight NEAAs (including preoviposition egg production from threonine-free diet fed flies) (Table 3). There were not significantly effect on survivorship and egg hatch (Table 4). Fecundity and survivorship from adults fed on the 2 diet free of any single NEAAs

Table 2. Effect of nonessential amino acids on larval development of *C. capitata*

Deleted amino acids	Larval period (d)	Pupal recovery (%)	Pupal weight (mg)	Adult emergence (%)	Flier (%)
None ( <i>C. capitata</i> 1 diet)	9.58d	80.50a	9.75a	96.69a	60.21a
Alanine	9.60d	83.50a	9.56ab	94.21a	68.24a
Aspartic acid	9.50d	70.50a	9.61ab	94.54a	61.33a
Cystine	9.64d	86.50a	9.93a	95.50a	78.19a
Glutamic acid	9.34d	83.00a	9.60ab	91.98a	71.57a
Glycine	15.78a	71.50a	8.72c	68.34b	78.03a
Proline	9.40d	62.50a	9.32abc	92.30a	59.64a
Serine	12.16b	80.50a	8.96bc	94.03a	75.55a
Tyrosine	9.24d	88.00a	9.88a	92.03a	75.07a
All 8 NEAA	11.34c	78.50a	9.33abc	88.88a	76.48a
F value	247.54	0.73	7.53	9.38	2.46
df	9, 30	9, 30	9, 30	9, 33	9, 33
P value	<0.0001	0.6775	<0.0001	<0.0001	0.0255

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

**Table 3. Summary of the effect of amino acids on mean fecundity**

Deleted amino acids	Mean fecundity Ratios (deleted/none)			
	Postoviposition		Preoviposition	
	Ratios	P value	Ratios	P value
None ( <i>C. capitata</i> 2)	1		1	
18 amino acids	0.29 ± 0.12	<0.0001	0.54 ± 0.12	0.0002
10 essential amino acids	0.46 ± 0.21	<0.0001	0.72 ± 0.21	<0.0001
8 nonessential amino acids	0.67 ± 0.17	<0.0001	0.82 ± 0.24	<0.0001
Arginine	0.74 ± 0.17	<0.0001	—	—
Histidine	0.78 ± 0.13	<0.0001	—	—
Isoleucine	0.81 ± 0.20	<0.0001	—	—
Leucine	0.65 ± 0.13	<0.0001	—	—
Lysine	0.88 ± 0.21	0.0012	—	—
Methionine	0.83 ± 0.21	<0.0001	—	—
Phenylalanine	0.85 ± 0.21	<0.0001	—	—
Threonine	0.42 ± 0.22	<0.0001	0.49 ± 0.22	<0.0001
Tryptophan	0.73 ± 0.13	<0.0001	—	—
Valine	0.68 ± 0.17	<0.0001	—	—
Alanine	1.04 ± 0.31	0.5220	—	—
Aspartic acid	1.11 ± 0.31	0.0673	—	—
Cystine	1.12 ± 0.26	0.0763	—	—
Glutamic acid	1.04 ± 0.24	0.4498	—	—
Glycine	1.00 ± 0.18	0.3388	—	—
Proline	1.06 ± 0.34	0.2841	—	—
Serine	1.03 ± 0.22	0.4650	—	—
Tyrosine	1.11 ± 0.22	0.0661	—	—

—, no data recorded.

remained normal compared with control diet (Table 5). Percentage of survivorship of males fed on the proline-free diet was reduced significantly compared with that from the control diet. Despite reduced fecundity in females fed on the EAA-free diet, sexual maturation was as normal as those from the control. These results coincide with the recent finding by Shelly and Kennelly (2002) that the addition of protein to the diet did not boost mating success of mass-reared males competing against wild or mass-reared males for wild females. The inclusion of protein in the male diet had no apparent effect on female remating tendency, copulation duration, or male longevity. Because the mating is not successful unless sexual maturity is reached, we conclude that amino acids are not fully responsible for sexual maturity.

Figure 2A shows that adults fed on both the test diets exhibited mature eggs, but the fecundity of flies fed with an EAA-free diet was significantly lower than that of the flies fed on the control diet. We speculate that amino acids might affect the morphogenetic pro-

cess in reproduction or the energy production during the oviposition as described by Chen (1985). Moreover, number of eggs in ovaries correlated well with the postoviposition eggs collected from the corresponding adults fed on the *C. capitata* 2 diet or the 10 EAA-free diet (Fig. 2B). The most significant age was shown for 10-d-old adults for egg production. The number of mature eggs in the ovaries was highest, whereas the postoviposition egg counts were the lowest during the 2-wk period. This could have also been caused by the inability of adults to find a suitable oviposition site. Our results show that there was significant reduced fecundity from all the 18 amino acids, 10 EAAs, or eight NEAA-free diet-fed adults, whereas other parameters were not significantly affected. Sexual maturity was still achieved in adult flies fed on the EAA-free diet. This is very different from the findings on *Bactrocera (Dacus) cucurbitae* by Kaur and Srivastava (1994) and Zografou et al. (1998). Kaur and Srivastava (1994) found that the 10 EAAs were indispensable, whether used singly or collectively, because

**Table 4. Effect of essential and nonessential amino acid groups on adult *C. capitata* reproduction**

Deleted amino acids	Mean postoviposition egg counts/female/day	% Survivorship		Egg hatch (%)
		Females	Males	
None ( <i>C. capitata</i> 2 diet)	22.23 ± 1.12a	85.60 ± 2.90ab	99.70 ± 0.30a	93.31 ± 0.92ab
10 Essential AA	12.16 ± 1.10b	86.40 ± 2.30ab	100.00 ± 0.00a	90.06 ± 1.18bc
8 Nonessential AA	13.56 ± 0.48b	93.90 ± 1.70a	98.10 ± 0.70a	89.88 ± 0.82bc
Threonine	7.29 ± 0.40cde	85.00 ± 2.43a	89.50 ± 1.96ab	—
Protein hydrolysate/sugar, 1:3	11.88 ± 2.36b	97.50 ± 0.70a	95.60 ± 1.20a	91.31 ± 0.75ab
All except sugar	0.87 ± 0.35c	64.40 ± 4.10c	82.20 ± 3.40b	91.63 ± 0.66ab
F value	25.01	12.46	20.53	8.45
df	5, 24	5, 280	5, 280	5, 120
P value	0.0001	0.0001	0.0001	0.0001

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

Table 5. Effect of individual nonessential amino acids on adult *C. capitata* reproduction

Deleted amino acids	Mean postoviposition egg counts/female/day	%Survivorship		Egg hatch (%)
		Females	Males	
None ( <i>C. capitata</i> 2)	11.59 ± 2.52a	84.69 ± 3.39ab	84.69 ± 3.08ab	11.59 ± 2.52a
All 8 nonessential AA	7.04 ± 0.39ab	83.75 ± 3.72ab	84.38 ± 2.58abc	7.04 ± 0.39ab
Alanine	8.12 ± 1.21ab	87.19 ± 2.26ab	97.19 ± 1.75a	8.12 ± 1.21ab
Aspartic acid	8.88 ± 1.81ab	75.31 ± 4.71ab	79.38 ± 5.04abc	8.88 ± 1.81ab
Cystine	9.58 ± 1.69a	91.56 ± 1.80a	95.31 ± 1.62a	9.58 ± 1.69a
Glutamic acid	11.35 ± 1.28a	90.31 ± 2.44a	87.81 ± 3.20ab	11.35 ± 1.28a
Glycine	11.30 ± 2.90a	76.88 ± 4.50ab	80.00 ± 5.75abc	11.30 ± 2.90a
Proline	13.17 ± 2.87a	71.56 ± 6.78b	65.31 ± 7.31c	13.17 ± 2.87a
Serine	13.96 ± 1.69a	85.63 ± 2.80ab	86.88 ± 2.99ab	13.96 ± 1.69a
Tyrosine	10.81 ± 0.55a	82.81 ± 3.21ab	79.69 ± 3.69abc	10.81 ± 0.55a
Protein:sugar, 1:3	6.12 ± 0.97ab	80.63 ± 2.54ab	84.69 ± 3.99ab	6.12 ± 0.97ab
F value	4.17	2.54	4.53	4.17
df	10, 36	10, 372	10, 372	10, 36
P value	0.0005	0.0041	0.0001	0.0005

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

in their absence the flies did not attain sexual maturity and produced no eggs. Additionally, the NEAAs as a group were essential, and their omission resulted in considerable reduction in longevity, total oviposition, and egg viability. Alanine, glutamic acid, or serine was found to be essential and aspartic acid, glycine, tyrosine, or cystine was beneficial, because their dele-

tion reduced longevity, total oviposition and egg viability. Asparagine, aminobutyric acid, ornithine, and cysteine could dispense without much adverse effect. Zografou et al. (1998) reported that insect survival of *B. oleae* was shortened by analogues of alanine, glutamine, cysteine, arginine, methionine, and proline, whereas fecundity was significantly reduced by the same analogs plus glycine, alanine, leucine, serine, glutamic acid, lysine, methionine, and histidine. They also reported that egg hatch was decreased with the exclusion of alanine, glutamic acid, cysteine, methionine, and histidine, whereas glutamic acid, methionine, tryptophan, histidine, and proline did not affect any of the test parameters. Our results for individual deletion of the 10 EAAs from *C. capitata* 2 diet indicated that the 10 EAAs were indispensable together or individually where NEAAs were not essential either individually or as a group for egg production. These discrepancies from other findings may be due to 1) metabolites inherited from the immature stages; 2) the role amino acid isomers play: some insect species may be more efficient than others in using minerals present in their diets (Anand and Anand 1994; Tsiropoulos 1977, 1978, 1980, 1983) than using amino acids; 3) the holidic diet we adopted may not be the optimal diet in terms of amino acid supplements; and 4) the possible effects of omitting certain amino acids on the efficacy and relative concentration of the remaining amino acids were not investigated. Further investigation on this aspect is warranted.

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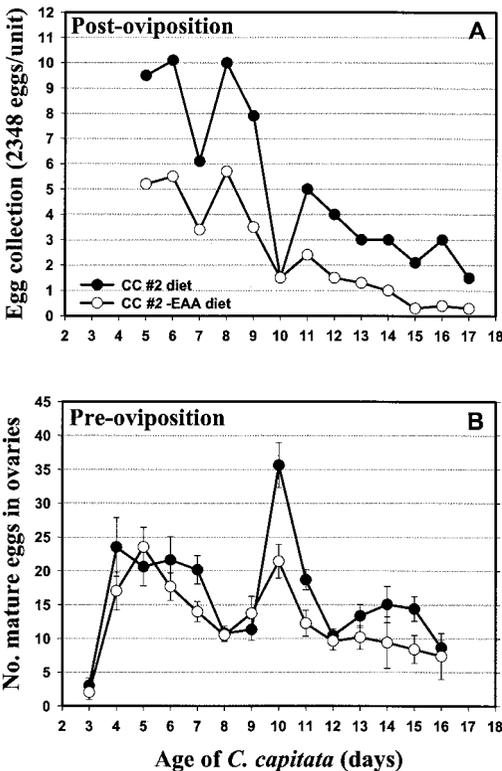


Fig. 2. Correlation between the postoviposition eggs (A) and preoviposition eggs (B) collected from adults fed on *C. capitata* 2 holidic adult diet or 2 diet without 10 EAAs. Both charts show that sexual maturity occurred even when adults were reared in essential amino acid-free diet.

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