ECOLOGY, BEHAVIOR AND BIONOMICS

Effects of Different Protein Concentrations on Longevity and Feeding Behavior of Two Adult Populations of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae)

Maria do Carmo Plácido-Silva[1], Alberto M. da Silva Neto[1], Fernando S. Zucoloto[2] and Iara S. Joachim-Bravo[1]

[1]*Depto. Biologia Geral, Instituto de Biologia, Univ. Federal da Bahia. Rua Barão do Geremoabo, s/n. Campus Universitário de Ondina, 40.170-290. Salvador, BA*
[2]*Depto. Biologia, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, USP, Av. Bandeirantes, 3900, Monte Alegre, 14.040-901, Ribeirão Preto, SP*

Neotropical Entomology 35(6):747-752 (2006)

Efeito de Diferentes Concentrações de Proteína na Longevidade e Comportamento Alimentar de Adultos de Duas Populações de *Ceratitis capitata* Wiedemann (Diptera: Tephritidae)

RESUMO - Neste estudo avaliou-se a influência da ingestão de proteína durante a fase adulta em machos e fêmeas de duas populações de *Ceratitis capitata* Wiedemann, uma composta por moscas criadas há vinte anos em laboratório sem a introdução de moscas selvagens (Lab-pop) e outra mantida há aproximadamente quinze anos com a introdução periódica destas (Hybrid-pop). Foram testadas três dietas: duas contendo levedura de cerveja (fonte protéica), respectivamente, nas concentrações de 6,5 g e 1,5 g por 100 ml de dieta e uma dieta sem levedura. Foram avaliados os parâmetros: longevidade dos adultos, ingestão de dietas com e sem levedura e limiar de discriminação para a levedura. A ingestão de proteína aumentou a longevidade dos adultos da Lab-pop, porém não influenciou a longevidade dos da Hybrid-pop. Dentro de cada população, ambos os sexos apresentaram longevidade similar, quando alimentados com o mesmo tipo de dieta. Adultos de ambas as populações apresentaram comportamento alimentar similar e preferiram se alimentar de dietas contendo fonte protéica (levedura) em relação a dietas sem essa fonte. Machos e fêmeas, em ambas as populações, ingeriram quantidades similares de uma mesma dieta. O limiar de discriminação para a levedura foi semelhante para os machos das duas populações (0,5 g/100 ml de dieta); as fêmeas da Lab-pop foram capazes de perceber uma quantidade menor de levedura na dieta, ou seja tiveram maior capacidade de discriminação (0,4 g/100 ml de dieta) em relação as fêmeas oriundas da Hybrid-pop (0,6 g/100 ml de dieta).

PALAVRAS-CHAVE: Mosca-das-frutas, nutrição, limiar de discriminação, dieta, sobrevivência

ABSTRACT - The effects of protein intake on two adult male and female populations of *Ceratitis capitata* Wiedemann were assessed. One population consisted of flies reared for twenty years in the laboratory (Lab-pop); the other population consisted both of flies reared in the laboratory for approximately fifteen years and of the periodically introduced wild flies (Hybrid-pop). Three diets were tested: a no-yeast diet and two diets containing yeast (protein source) at the concentrations 6.5 g or 1.5 g per 100 ml diet. The parameters analyzed were: adult longevity, diet intake with and without yeast, and discrimination threshold for yeast. Protein intake increased Lab-pop adult longevity and did not affect longevity of the Hybrid-pop. Longevity in each population was similar for males and females fed on the same diet. Food behavior were similar for male and female adults of both populations; all preferred diets containing protein (yeast). Males and females in both populations ingested similar amounts of each diet. The discrimination threshold for yeast was similar for all males (0.5 g yeast/100 ml diet); Lab-pop females were able to detect the presence of smaller quantities of yeast in their diet, thus having a higher discrimination capacity (0.4 g/100 ml diet) as compared to the Hybrid-pop females (0.6 g/ 100 ml diet).

KEY WORDS: Fruit fly, nutrition, discrimination threshold, diet, survival

Ceratitis capitata Wiedemann, known as Mediterranean fruit fly, is an important fruit pest around the world. For this reason, it has been reared systematically in the laboratory for basic and applied research purposes. The maintenance of insect colonies under artificial conditions requires that the ecological, behavioral, and genetic characteristics of insects be similar to those of wild insects (Boller & Chambers 1977). However, several studies have shown changes in the behavior and in some physiological and demographic characteristics of insect populations reared in the laboratory, such as birth, mortality, and fecundity rates (Leppla *et al*. 1983, Economopoulos 1992, Joachim-Bravo & Zucoloto 1998). Laboratory strains usually reach maturity earlier and have higher reproduction rates but lower flight ability (Leppla *et al*. 1983) than wild ones. Important behavioral differences between wild and laboratory *C. capitata* populations also were found, such as those concerning the ability of the immature to select food and the capacity of females to discriminate hosts (Joachim-Bravo & Zucoloto 1998).

The characteristics of laboratory strains are probably altered due to selection caused by drastic changes from natural to artificial rearing conditions, particularly by the introduction of an artificial diet and the high frequency of endogamy (Economopoulos 1992). Several strategies have been used to lower the impact of these problems, such as rearing conditions that promote relaxation to recover lineage, hybridization with compatible lineages, and colony supply with wild individuals (Leppla 1989, Zucoloto 2000). The latest procedure is frequently used, particularly in small scale rearing for the maintenance of genetic variability in populations.

The goal of this study was to find out if a laboratory population receiving wild individuals responded to variations in food nutritional quality the same way as a laboratory population reared exclusively in the lab. The effects of different protein concentrations on male and female longevity and food selection behavior (diet with or without protein) were assessed. As known, protein is the most important nutrient for adult survival and reproduction.

Materials and Methods

The two *C. capitata* populations studied were reared in the laboratory. One of them was reared since 1980 (Lab-pop) and the other, for the last fifteen years and received wild flies at least once a year (Hybrid-pop). For the period of one year before the experiments started, approximately 10% of wild individuals were brought monthly to the laboratory. During the research (approximately two months) wild flies were not brought to the lab, to prevent their use in the experiments.

The populations were maintained under laboratory conditions according to Zucoloto (1987). Adults received daily amounts of water and an artificial diet containing 6.5 g yeast (Mãe-Terra, Mãe-Terra Produtos Naturais Ltda.), 11.0 g sugar (União, União de Refinadores do Brasil), 2.0 g agar (Isofar, Isofar Indústria e Comércio de Produtos Químicos Ltda.), 1.0 g citric acid (Vetec, Vetec Química Fina Ltda.), 1.0 g nipagin (Isofar), and 100 ml distillled water (Zucoloto *et al*. 1979).

To assess longevity, newly emerged adults of each population were separated into three groups, each group containing 20 males and 20 females. Each group was given the diet described above, with differing yeast concentrations (protein source): 6.5 g or 1.5 g/ 100 ml diet). The control diet did not contain yeast. Male and female adults of each group were kept separately, in groups of five individuals placed in plastic cages (10 x 5 cm) with lateral orifices. Small chunks of food were pinned to a cork (± 1 cm diameter) and placed into the pots through an orifice (Cangussu & Zucoloto 1992). Water was provided in small assay tubes tamponed with cotton and inserted through a different orifice. Food and water were replaced daily and dead individuals were removed from the cages.

Longevity data was analysed by 3-way ANOVA and the statistical program GMAV.5 for Windows (University of Sidney, Underwood & Chapman 1997). Multiple comparisons of means were conducted by the test Student-Newman-Keuls (SNK). The Cochran's test was used to test variance homogeinity. Whenever data transformation could not remove variance heterogeinity, the non-transformed data were still used due to design strength – a large sample (n = 20) and the same sample size for all groups (Underwood 1997).

Diet selection (with or without protein) by adult males and females in both populations was conducted according to Cangussu & Zucoloto (1995). Fifteen newly emerged females and males in each population were placed in an acrylic box (11 x 11 x 3 cm), being each box considered an experimental unit. Each unit received 0.4 g of two diets daily: one contained saccharose and water; the other, yeast + saccharose and water. Metilparabene (1 ml/ 100 ml diet) was added to all diets to prevent fermentation. Evaluation of water loss by evaporation was conducted by keeping one box with water and the diet. Diets were replaced daily and at the same time, and placed in a sterilization chamber at 50°C for 24h for drying. After the 24h needed to reach constant weight, the food was weighed and ingestion per box was calculated. Daily diet intake per box was calculated following Cangussu & Zucoloto (1995):

$$I = \frac{TDM - RDM}{N}$$

where: I = ingestion; TDM = total dry matter (box without flies); RDM = remaining dry matter (experimental box); N = number of live flies.

Ingestion was assessed for five consecutive days. During this period, dead flies were daily removed to prevent error in ingestion calculations. Ten replicates were conducted for each sex and population. The paired t-test was used (after normality correction with data logarythm transformation) with the support of the statistical program SPSS, version 12.0 (SPSS, Inc.).

Estimates of adult capacity to discriminate protein quantity in the diets of both populations were done by evaluating the discrimination threshold for yeast (protein source). The threshold was assessed by ingestion measuring of diets containing different yeast concentrations. Diets containing decreasing yeast concentrations (starting at 0.5 g yeast/ 100 ml diet) were compared with diets without yeast, in free-choice tests between the two diets (with and without yeast). Ingestion data were taken for three consecutive days (diets were replaced every 24h), according to the procedures

Table 1. Summary of the 3-way ANOVA results for analysis of the effect of population, diet yeast concentration, and sex on *C. capitata* adult longevity.

	DF	MS	F	P
Population (Pop)	1	1926.6667	11.85	0.0007
Yeast Concentration (YC)	2	1313.3292	8.08	0.0004
Sex (Se)	1	968.0167	5.95	0.0154
Pop x YC	2	2539.1292	15.62	0.0000
Pop x Se	1	64.0667	0.39	0.5308
YC x Se	2	30.9292	0.19	0.8269
Pop x YC x Se	2	185.1292	1.14	0.3220
RESIDUAL	228	162.5711		

described in the previous experiment. This test consisted of three replicates for each yeast concentration tested per sex and population. Data analysis was conducted using the paired t-test (after correction for normality with data logarythmic transformation).

Results

Analysis of the impact of population, yeast concentration, and sex on adult longevity showed an interaction between population and yeast concentration (Table 1). Populations responded differently to food quality. Yeast-enriched diets increased Lab-pop adult longevity but did not affect Hybrid-pop adults (Fig. 1, Table 2).

In all treatments, Lab-pop adults fed on diets containing 1.5 g and 6.5 g yeast lived longer than Hybrid-pop adults. Without the protein, however, longevity was greater for the Hybrid-pop population (Fig. 1, Table 2).

Sex and protein concentration were not related statistically and adults of both sexes responded similarly to the effect of yeast concentration on longevity (Table 1). However, females of both populations and fed on any of the diets lived longer than males (Fig. 1, Table 2).

The analysis of adult feeding behavior in diets with and without protein revealed that individuals of both sexes in the two populations ingested greater amounts of food containing yeast + saccharose than food with saccharose only (Table 3). There was no difference between food intake by male and female flies in both populations.

The minimum concentration of yeast discriminated by males of both populations was 0.5 g /100 ml diet, showing the greater intake of this diet as compared to the diet without yeast. Below this concentration, males ingested similar amounts of food, with or without yeast. Mean ingestion of the minimum yeast concentration and of the immediately lower yeast concentration are shown in Table 4. Therefore, adult discrimination threshold for yeast in both populations was established between 0.4 g and 0.5 g yeast per 100 ml diet. As opposed to male behavior, females differed in their discrimination threshold for yeast according to population. Lab-pop females discriminated their minimum yeast concentration at 0.4 g / 100 ml diet and a threshold defined between 0.3 g and 0.4 g yeast per 100 ml diet. Hybrid-pop females discriminated a minimum yeast concentration of 0.6 g per 100 ml diet, the threshold being between 0.5 g and 0.6 g per 100 ml diet (Table 4).

Discussion

In this study, the population reared in the laboratory without wild flies (Lab-pop) used the protein diet more efficiently and consequently, had greater longevity than the

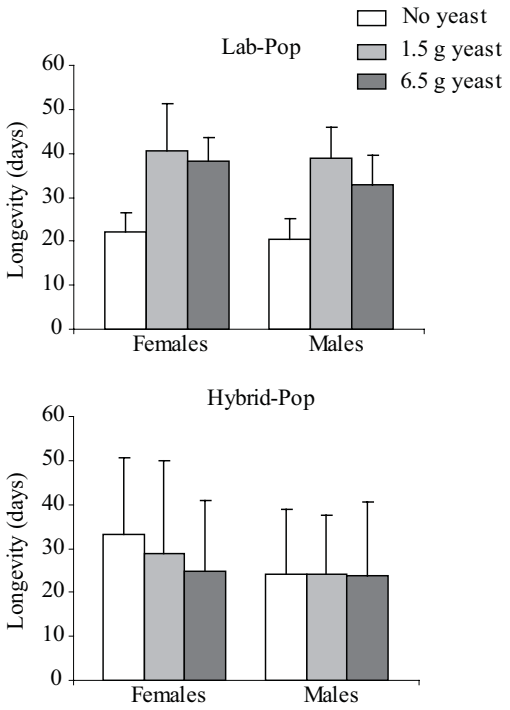

Fig. 1. Effects of adult ingestion of different protein (yeast) concentrations on longevity of adult females and males of two *C. capitata* populations. Results represent mean and standard-deviation values for 30 individuals of each sex and population.

Table 2. Summary of results of the test Student-Newman-Keuls (SNK) for comparison of factors affecting *C. capitata* adult longevity.

Yeast concentration x population (YC x Pop)	
	P
YC0,0: Lab-Pop < Hybrid-Pop	*
YC1,5: Lab-Pop > Hybrid-Pop	**
YC6,5: Lab-Pop> Hybrid-Pop	**
Lab-Pop: YC0,0 < YC1,5 = YC6,5	**
Hybrid-Pop: YC0,0 = YC1,5 = YC6,5	NS
Sex	
Female > male	*

* = P < 0.05, ** = P < 0.01. (YC 0.0 = no yeast, YC1.5 = 1.5 g yeast, YC6.5 = 6.5 g yeast)

laboratory population receiving wild flies (Hybrid-pop). This can be considered a direct impact of the better Lab-pop adaptation to the artificial diet containing yeast, considering that previous studies have shown that the artificial diet is one of the main determinants of population selection in the laboratory (Leppla *et al.*1983, Leppla 1989).

The Lab-pop fed on the no-yeast diet had lower longevity than the Hybrid-pop, which suggests that the former is more sensitive to the lack of protein in adulthood. Assuming that Hybrid-pop characteristics are closer to those of the natural population, the difference between Lab-pop and Hybrid-pop can be interpretated as an adaptive strategy of natural populations that is lost under laboratory colonization conditions. In natural environments, protein resources are less abundant for phytophagous species than energetic resources (carbohydrates) and foraging costs for protein are higher; then, if natural populations were more sensitive to the lack of protein, it would affect their survival and reproduction importantly. Colonization under laboratory conditions with protein abundance can lead to the loss of this adaptive characteristic and also to selective pressure for a more efficient protein use, which can bring changes to the life history of natural populations.

Results of studies on the effect of protein on insect longevity have been controversial. The addition of yeast to adult diet lowered longevity and increased posture as a "trade-off" due to the reproductive cost in *C. capitata* (Carey *et al.* 1998). Cangussu & Zucoloto (1997), on the other hand, observed a lower longevity when protein was not offered to insects during the adult phase. For other insects such as *Drosophila melanogaster* Meiem (Diptera: Drosophilidae), protein-enriched diets increased adult life span (Good & Tatar 2001). According to them, protein is not essential for *D. melanogaster* survival although it would be required for optimum longevity. Our data support other studies that show the importance of protein for adult longevity.

Contrary to our expectations, males and females responded similarly to protein ingestion and its effect on longevity. In general, females lived longer than males although no relationship was established between sex and diet quality. However, Muller *et al*. (1997) found that females lived longer than males when both were fed on protein diets. Considering the important role of protein for egg production, we expected that females fed on the no-protein diet would live less than males reared under the same conditions, given that previous studies have shown a higher reproductive cost for females than for males, and lower female longevity (Chapman *et al*. 1995, 1998). Our results do not support those obtained by Chapman *et al*. (1995, 1998), but corroborate with findings of Chang *et al*. (2001), where protein affected male and female longevity equally.

Adults in each population responded differently to the effect of protein on longevity but did not differ in consumption levels. All adults had similar selective feeding behavior, always preferring the protein-enriched diet. These results suggest that in a colonization process in the laboratory, behavioral changes concerning the acceptance of a high-protein artificial diet can occur sooner than physiological changes such as those related to metabolism and diet use.

Female preference for diets containing protein was expected. As highlighted in previous studies, protein is crucial for egg production (Robacker 1991, Cangussu & Zucoloto 1993, Ashworth & Wall 1995). However, this same result was unexpectedly also found for males. During the immature phase of *C. capitata* and other organisms, protein consumption by males affects their size and copulation success (Blay & Yuval 1997). The role of protein ingestion during adulthood in male reproductive performance is

Table 3. Adult male and female diet ingestion (mg/day) for two *C. capitata* populations.

Population		Diet		P
		Yeast + saccharose	Saccharose	
Lab-pop	Males	0.80 ± 0.1866a	0.31 ± 0.1039b	< 0,0001
	Females	0.88 ± 0.1857a	0.31 ± 0.0885b	< 0,0001
Hybrid-pop	Males	0.73 ± 0.1206a	0.32 ± 0.0713b	< 0,0001
	Females	0.74 ± 0.1115a	0.35 ± 0.0752b	< 0,0001

Results represent the mean and standard deviation values for ten replicates of each sex and population. Mean values followed by different letters in the row differ statistically (paired t-test at 5% significance).

Table 4. Discrimination threshold for yeast.

Groups	Yeast concentration (g/100 ml water)	Ingestion (mg/day) of diets containing yeast	Ingestion (mg/day) of no-yeast diets	P
Lab-pop – males	0.5	0.678 ± 0.152a	0.544 ± 0.080b	0.0305
	0.4	0.506 ± 0.083a	0.508 ± 0.075a	0.8600
Lab-pop – females	0.4	0.523 ± 0.063a	0.327 ± 0.078b	0.0013
	0.3	0.631 ± 0.112a	0.509 ± 0.124a	0.0743
Hybrid-pop – males	0.5	0.415 ± 0.0001a	0.260 ± 7.8880b	0.0095
	0.4	0.390 ± 9.7350a	0.380 ± 7.8770a	0.8416
Hybrid-pop – females	0.6	0.560 ± 0.0001a	0.390 ± 6.5230b	0.0053
	0.5	0.458 ± 0.0001a	0.342 ± 0.0001a	0.0835

Results represent the mean and standard-deviation values for quantity of the diet ingested by ten replicates of each sex and population. For each group, mean ingestion of the minimum concentration of discriminated yeast and its immediately lower concentration (non-discriminated) were compared to no-yeast diets. Mean values (mg/day) followed by different letters in the row differ statistically (paired t-test at 5% significance).

still controversial. Protein can affect the production of spermatozoids but results of studies on the importance of protein in male copulation success are still contradictory (Shelly *et al*. 2002, Shelly & McInnis 2003).

As opposed to diet selection tests, results of threshold for yeast discrimination revealed differences between the adults of the populations tested. Lab-pop females were able to discriminate a smaller quantity of yeast than Hybrid-pop females. Apparently, this difference means that Lab-pop females are more sensitive to the presence of protein, probably because the nutrient was abundantly offered to them. According to Cresoni-Pereira & Zucoloto (2001), there can be a relationship between the availability of a given nutrient and the threshold for its discrimination. Discrimination threshold for more abundant nutrients would be lower. Laboratory flies tend to have higher fecundity than wild flies (Leppla *et al*. 1983), which can lead to a greater protein need by the former. Consequently, laboratory populations can need more protein than wild populations. Hybrid population males discriminated protein at lower quantities than females and although the difference was small (0.1 g/ 100 ml), it can be representative. Male protein needs during the first three or four days of adult life can be a little higher as compared to females because these days correspond to the male sexual maturation period.

In our study, males and females responded similarly to the tested parameters, independently of the population to which they belonged. However, populations reared only in the laboratory and for long periods can behave and biologically perform differently from lab-populations receiving wild flies. Therefore, research results of populations reared only in the laboratory must be carefully interpreted and further studies should compare hybrid and exclusively wild populations.

Acknowledgment

We thank CNPq for supporting the research (Grant 475050/2001-0) and Francisco Barros, for his contribution in the statistical analysis.

References

Ashworth, J.R., R. Wall. 1995. Effects of ovarian development and protein deprivation on the activity and locomotor responses of the blowfly, *Lucilia sericata*, to liver odour. Physiol. Entomol. 20: 281-285.

Blay, S. & B. Yuval. 1997. Nutritional correlates of reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). Anim. Behav. 54: 59-66.

Boller, E.F. & D.L. Chambers. 1977. Quality aspects of mass-reared insects, p.219-235. In R.L. Ridgw & S.B. Vinson (eds.), Biological control by augmentation of natural enemies. Plenum Press, New York, 480p.

Cangussu, J.A. & F.S. Zucoloto. 1992. Nutritional value and selection of different diets by adult *Ceratitis capitata* fruit flies. J. Insect Physiol. 38: 485-491.

Cangussu, J.A. & F.S. Zucoloto. 1993. Influence of partial malnutrition on egg production by *Ceratitis capitata* (Diptera, Tephritidae). Rev. Bras. Biol. 53: 155-158.

Cangussu, J.A. & F.S. Zucoloto. 1995. Self-selection and perception threshold in adult females of *Ceratitis capitata*. J. Insect Physiol. 41: 223-227.

Cangussu, J.A. & F.S. Zucoloto. 1997. Effect of protein sources on fecundity, food acceptance and sexual choice by *Ceratitis capitata* (Diptera, Tephritidae). Rev. Bras. Biol. 5: 611-618.

Carey, J.R., P. Liedo, H.G. Muller, J.L. Wang & J.W. Vaupel. 1998. Dual modes of aging in Mediterranean fruit fly females. Science 281: 996-998.

Chang, C.L., C. Albrecht, S.S.A. El-Shall & R. Kurashima. 2001. Adult reproductive capacity of *Ceratitis capitata* (Diptera, Tephritidae) on a chemically defined diet. Ann. Entomol. Soc. Am. 94: 702-706.

Chapman, T., L.F. Liddle, J.M. Kalb, M.F. Wolfner & L. Partridge. 1995. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. Nature 373: 241-244.

Chapman, T., T. Miyatake, H.K. Smith & L. Partridge. 1998. Interactions of mating, egg production and death rates in females of the Mediterranean fruit fly, *Ceratitis capitata*. Proc. R. Soc. Lond. B Biol. Sci. 265: 1879-1894.

Cresoni-Pereira, C. & F.S. Zucoloto. 2001. Dietary self-selection and discrimination threshold in wild *Anastrepha obliqua* females (Diptera, Tephritidae). J. Insect Physiol. 47: 1127-1132.

Dadd, R.H. 1985. Nutrition: Organisms, p.313-389. In S.A. Kerkut & J.F. Gilbert (eds.), Comprehensive insect physiology, biochemistry and pharmacology, vol. 4, Pergamon Press, Oxford, 743p.

Economopoulos, A.P. 1992. Adaptation of the Mediterranean fruit fly (Diptera; Tephritidae) to artificial rearing. J. Econ. Entomol. 85: 753-758.

Good, T.P. & M. Tatar. 2001. Age-specific mortality and reproduction respond to adult dietary restriction in *Drosophila melanogaster*. J. Insect Physiol. 47: 1467-1473.

Joachim-Bravo, I.S. & F.S. Zucoloto. 1998. Performance and feeding behavior of *Ceratitis capitata*: Comparison of a wild population and a laboratory population. Entomol. Exp. Appl. 87: 67-72.

Leppla, N.C. 1989. Laboratory colonization of fruit flies, p.91-103. In A.S. Robinson & G. Hooper (eds), Fruit flies: Their biology, natural enemies and control, vol. 3B, Elsevier, New York, 447p.

Leppla, N.C., M.D. Huettel, D.L. Chambers, T.R. Ashley, D.H. Miyashita, T.T.Y. Wong, E.J. Harris. 1983. Strategies for colonization and maintenance of the Mediterranean fruit fly. Entomol. Exp. Appl. 33: 89-96.

Muller, H.G., J.L. Wang, W.B. Capra, P. Liedo & J.R.Carey. 1997. Early mortality surge in protein-deprived females causes reversal of sex differential of life expectancy in Mediterranean fruit flies. Proc. Natl. Acad. Sci. U. S. A. 94: 2762-2765.

Robacker, D.C. 1991. Specific hunger in *Anastrepha ludens*: Effects on attractiveness of proteinaceous and fruit-derived lures. Environ. Entomol. 20: 1680-1686.

Shelly, T.E. & D.O. McInnis. 2003. Influence of adult diet on the mating success and survival of male Mediterranean fruit flies (Diptera: Tephritidae) from two mass-rearing strains on field-caged host trees. Fla. Entomol. 86: 340-344.

Shelly, T.E., S.S. Kennelly & D.O. McInnis. 2002. Effect of adult diet on signaling activity, mate attraction and mating success in male Mediterranean fruit flies (Diptera: Tephritidae). Fla. Entomol.85: 150-155.

Underwood, A.J. 1997. Experiments in ecology: Their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge, 496p.

Underwood, A.J. & M.G. Chapman. 1997. Statistical program GMAV.5 for Windows. Institute of Marine Ecology, University of Sidney, Australia.

Zucoloto, F.S. 1987. Feeding habits of *Ceratitis capitata*: Can larvae recognize a nutritional effective diet? J. Insect Physiol. 33: 349-353.

Zucoloto, F.S. 2000. Alimentação e nutrição de moscas-das-frutas, p.67-80. In A. Malavasi & R.A. Zucchi (eds.), Moscas-das-frutas de importância econômica no Brasil: Conhecimento básico e aplicado, Holos, Ribeirão Preto, 327p.

Zucoloto, F.S., S. Puschel & C.M. Message. 1979. Valor nutritivo de algumas dietas artificiais para *Anastrepha obliqua* (Diptera: Tephritidae). Bol. Zool. Univ. S. Paulo 4: 75-80.

Received 12/V/05. Accepted 3/V/06.