

EFFECT OF ADULT DIET ON LONGEVITY OF STERILE MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Fly longevity is critical to sterile release programs for Mediterranean fruit flies (medflies) because the longer sterile flies are present after a release, the greater the probability of mating. Current release programs provide sterile, adult medflies with sucrose in an agar matrix for 2-3 d before release. We used cages to compare the effects of different diets on the longevity of medfly, *Ceratitis capitata* (Wiedemann). A diet of dry hydrolyzed yeast + sucrose supplied during the pre-release interval did not significantly affect field survivorship of medfly adult males relative to the standard sucrose diet. Post-release diets, simulating nitrogen and sugar sources that released medflies may find after release, had significant effects on medfly survivorship. Hydrolyzed yeast + sucrose resulted in the highest medfly survivorship, followed by sucrose, and then water alone. Finally, diets containing hydrolyzed yeast were not found to have significant amounts of protein and thus are more likely nitrogen or amino acid sources for flies, rather than sources of protein.

Key Words: *Ceratitis capitata*, sterile insect technique, longevity, hydrolyzed yeast, protein

RESUMEN

La longevidad de las moscas es crítica en un programa de técnicas de insecto estéril para la mosca mediterránea de la fruta porque el mas tiempo que las moscas estériles esten presentes después de ser liberadas, mayor es la probabilidad del apareamiento. Los programas actuales de liberación de adultos estériles de la mosca mediterránea los proveen con sucrosa en una matriz de agar por 2-3 días antes de su liberación. Nosotros usamos jaulas para comparar los efectos de dietas diferentes sobre la longevidad de la mosca mediterránea *Ceratitis capitata* (Wiedemann). Una dieta de levadura seca hidrolizada + sucrosa suplida durante el intervalo pre-liberación no tuvo un efecto significante sobre la sobrevivencia de los machos adultos de la mosca mediterránea en el campo en relación con la dieta estándar de sucrosa. Las dietas de pos-liberación, que simulan las fuentes de nitrógeno y azucar que la mosca mediterránea liberada y que pueden ser encontradas después de ser liberadas, tuvo efectos significantes sobre la sobrevivencia de las moscas mediterránea. La levadura seca hidrolizada con sucrosa resulta en una sobrevivencia lo mas alta de mosca mediterránea, seguido en orden por la dieta de sucrosa y después solo el agua. Por último, las dietas que contienen levadura hidrolizada no tuvieron cantidades significantes de proteína y por lo que probablemente son fuentes de nitrógeno o de aminoácidos para las moscas, al contrario de ser fuentes de proteína.

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), is a multivoltine, polyphagous insect pest that could have a devastating economic impact if it were to become established in California or Florida (Robinson et al. 1986; Liquido et al. 1991; Metcalf 1994). Presently, the sterile insect technique (SIT) is used in these states as a preventative measure to reduce the potential for Mediterranean fruit fly colonization. For wild males, reproductive success is the end result of the following steps: joining a lek, pheromone emission, courtship behavior, copulation, sperm transfer and storage, fertilization of eggs, and preventing/delaying female re-mating (Yuval et al. 2002). The goal of SIT is for released sterile

males to mate with any introduced wild females, resulting in the production of infertile eggs (Knippling 1955). Sterile male longevity after release and competitiveness with wild males are two factors that can impact the effectiveness of a SIT program.

Protein and carbohydrates are necessary dietary components for optimum medfly development and fecundity and could impact the effectiveness of sterile males. In the field, tephritid fruit flies have a larval diet that is often protein-deficient; whereas the larval diet of laboratory-reared, sterile flies is protein-rich (Cayol 2000). Cangussu and Zucoloto (1997) found that larvae consuming a protein-rich diet followed by a pro-

tein-poor adult diet resulted in females capable of producing more eggs than females having a protein-poor larval diet followed by a protein-rich adult diet. For adults, field sources of carbohydrates include injured fruits and honeydew, and proteins are commonly found in bird feces and rotting fruit colonized by bacteria (Hendrichs & Hendrichs 1990; Hendrichs et al. 1991).

Many studies have characterized the effects of protein-rich and protein-deficient adult diets (i.e., diets containing protein + sugar vs. sugar only, respectively) on wild and laboratory medflies (Yuval et al. 2002; Yuval et al. 2007). Laboratory strains of medfly fed protein-deficient adult diets sometimes showed an overall reduction in longevity (Cangussu & Zucoloto 1997; Niyazi et al. 2004), or had a comparable longevity to protein-fed flies (Shelly & Kennelly 2002; Shelly & McInnis 2003). In contrast, Kaspi & Yuval (2000) found 24 h of starvation resulted in greater mortality for protein-fed than protein-starved laboratory strain flies. Protein-fed wild flies and laboratory males were more likely to emit pheromones in leks than protein-starved flies (Papadopoulos et al. 1998; Kaspi et al. 2000; Kaspi and Yuval 2000). Yuval et al. (1998) found that field-collected lekking wild males contained higher amounts of protein and sugar compared to resting males. From these studies it is clear that there are some benefits of protein in the adult diet, however the impact would often depend on field conditions (e.g., likelihood of 24 h starvation).

Mating success of medflies can also be impacted by diet. Laboratory strain males provided with protein were 1.4-2 \times more likely to mate than protein-starved males (Taylor & Yuval 1999; Kaspi et al. 2000; Kaspi & Yuval 2000; Shelly et al. 2002), but in other studies no effects of diet were found (Shelly & Kennelly 2002; Shelly & McInnis 2003). Niyazi et al. (2004) reported that laboratory flies provided with a pro-biotic, yeast-enhanced, sugar diet had a significant mating advantage over protein-starved flies fed either a pro-biotic or non-probiotic diet in the laboratory, but there was no significant effect of diet between these 2 treatments in field cage studies. In one study with wild flies, the inclusion of protein in the adult diet increased mating success over protein-starved counterparts (Shelly & Kennelly 2002).

In part due to the high mortality of sterile medflies in the field, releases of sterile medflies occur twice a week in the Preventative Release Program in California (Barry et al. 2002). Sterile adult medflies are provided with sucrose in agar (1 M sucrose, no protein) 2-3 d before release in SIT programs such as that in California. It is possible that sterile males may not find needed nutrients following field release to allow for adequate longevity and reproductive development so as to mate with wild females. However, in a study with field enclosures, Maor et al. (2004) reported that

both protein-starved and protein-fed flies were able to successfully forage for protein and sugar when it was available. Improving the pre-release adult diet is one possible method of increasing SIT fly longevity in the field.

Kaspi and Yuval (2000) reported that the inclusion of protein hydrolysate in pre-release diets increased mating success while reducing longevity. In California, the inclusion of hydrolyzed yeast into the agar matrix of SIT medflies resulted in a high bacterial population forming on the diet media, and as a result, the concentration of an antimicrobial compound (methyl paraben, C₈H₈O₃) was increased, which subsequently lowered fly survivorship (R. V. Dowell, Calif. Dept. of Food & Agric., pers. comm.).

In this paper we investigated the effects of pre- and post-release diets on sterile medfly longevity in small field cages (without plants), and determined the levels of protein that were provided by different diets containing hydrolyzed yeast.

MATERIALS AND METHODS

Source of Insects

Sterile medfly puparia (Vienna-7, laboratory strain) were obtained in Oct of 2002, from the USDA/C DFA Cooperative Mediterranean fruit fly Preventative Release Program. The medflies for our tests were reared at the California Department of Food and Agriculture (C DFA) larval rearing center in Waimanalo, Hawaii. Puparia were dyed with Day-Glo neon red dye (Day-Glo Color Corp., Cleveland, OH), at 3 g of dye per liter of medflies, using a mechanical mixer containing 10-20 L of puparia (D. McInnis, USDA-ARS, pers. comm.). The puparia were tumbled gently for a few minutes to dye all of the flies prior to irradiation at 8 d of puparial age. Two days prior to eclosion, puparia were placed in hypoxia for 1-2 h and irradiated with a dose of 145 Gy from a Cobalt⁶⁰ pool-type irradiator. Puparia were subsequently shipped to the David R. Rumsey Emergence and Release Facility in Los Alamitos, California.

Pre-release and Post-release Diets

Survivorship studies were conducted in Riverside, CA with sterile medflies to simulate treatment of adult male medflies used in the SIT release program in California, which are held and fed for 2-3 d before being released into the environment.

Two pre-release diet treatments were offered to newly eclosed medflies for 48 h: (1) sucrose in agar, which is the standard medfly diet used in the CA preventative release program (94.98% sucrose, 4.95% agar, and 0.07% methyl paraben in 1 L of distilled, sterile water per 181 g of dry matter) (Niyazi et al. 2004), or (2) sucrose in agar (as in diet #1), with the addition of a separate dry mix-

ture of hydrolyzed yeast + dry sucrose (1:3, wt:wt). For the first 48 h, flies were held at the David R. Rumsey Emergence and Release Facility. After 48 h, groups of 20-30 flies, each in a 0.5-L plastic container were transferred in coolers by vehicle to Riverside, CA (approximately 2.5 h in transit).

One group of flies was randomly selected to be placed into each of 80 small field cages (without plants; $30.5 \times 30.5 \times 30.5$ cm³; Bioquip, Rancho Dominguez, CA), located along a southwest-facing wall, underneath a cover (6 m \times 3 m) to limit exposure to the wind and sun. The following 4 post-release diet treatments were offered to flies: (1) 10 g hydrolyzed yeast + dry sucrose (1:3 by weight) + water, (2) 10 g dry sucrose + water, (3) water, or (4) nothing (i.e., no food or water provided). Water was provided in a 20-mL container with a cotton wick and was changed every 4-5 d. Dry hydrolyzed yeast and sucrose mixtures were provided in plastic dishes (and were not mixed with water). A total of 10 replicate cages with 20-30 adult males per cage were used for each medfly diet treatment (2 pre-release \times 4 post-release treatments).

Cages were checked daily for fly mortality, and, after all flies had died, the average longevity of flies was determined for each cage. At the cage site, the average high temperature was 29.7°C, average low temperature was 12.7°C, and average percent relative humidity was 61%.

Statistical Analyses

One-way and two-way Analysis of Variance (ANOVA) was used to compare square root-transformed ($\sqrt{x + 0.5}$) data of the average medfly longevity per cage for pre-release and post-release diets, with means of significant factors separated by Fisher's Least Significant Difference (LSD) test (Minitab, Inc. 1998).

Protein Analysis

Protein levels were determined by a modified Bradford method developed by Bio-Rad (Bio-Rad Protein Assay, catalog #500-0002) (Bradford 1976).

Hydrolyzed yeast (Fisher Scientific, BP1422-500) was dissolved in de-ionized water at a concentration of 1mg yeast per 1 mL water solution. Seven samples of this solution were prepared, each in triplicate. Standard protein curves were established with 3 concentrations of protein standard. Bovine serum albumin (1.46 mg/mL) was diluted with de-ionized water to concentrations of 10 μ g/mL, 5 μ g/mL, and 2.5 μ g/mL. Three replicates of all samples and standards were assayed for protein content. An 800-mL aliquot of sample or standard was placed in a clean test tube to which 200 mL of reagent was added (Bio-Rad Protein Dye Reagent catalog #500-0006) and allowed to sit for 5 minutes before reading in a spectrophotometer at 595 nm. A "blank" consisting of 800 μ L de-ionized water plus 200 μ L reagent was tested. The absorbance values for the 3 replicates of each sample were averaged for the final value. The final values were compared to the standard curve to determine the amount of protein present in the hydrolyzed yeast. Protein values are presented as micrograms of protein per milligram of yeast.

RESULTS

Pre-release and Post-release Diets

Adult medfly longevity was significantly affected by post-release diet, but was not affected by pre-release diet or the interaction of pre- and post-release diet (Table 1; Pre-release $F = 3.52$; $df = 1$; $P = 0.065$; Post-release: $F = 184.80$; $df = 3$; $P < 0.0001$; Interaction: $F = 0.99$; $df = 3$; $P = 0.401$; Error: $df = 72$). When data for pre-release diets were pooled, post-release diet significantly affected adult medfly longevity (Fig. 1; $F = 178.92$; $df = 3, 76$; $P < 0.0001$). Males that were offered the post-release hydrolyzed yeast + sucrose + water diet lived significantly longer than those offered all 3 other post-release diets, and males provided the sucrose + water diet were significantly longer lived than flies offered water and flies offered nothing. At the end of the trial, there was still excess hydrolyzed yeast and sucrose mixture left in those cages where it was provided.

TABLE 1. LONGEVITY OF ADULT MALE MEDFLIES EXPOSED TO DIFFERENT POST-RELEASE DIET REGIMENS.

Pre-release diet (0-48 h)	Post-release diet (>48 h)	Longevity after 48 h (mean days \pm SE)
Sucrose+agar	No food or water	3.1 \pm 0.08
	Water	3.6 \pm 0.05
	Sucrose + water	10.9 \pm 0.70
	Sucrose/yeast hydrolysate + water	13.5 \pm 1.00
Sucrose + agar + yeast hydrolysate	No food or water	3.2 \pm 0.05
	Water	3.7 \pm 0.07
	Sucrose + water	12.0 \pm 0.90
	Sucrose/yeast hydrolysate + water	14.7 \pm 0.80

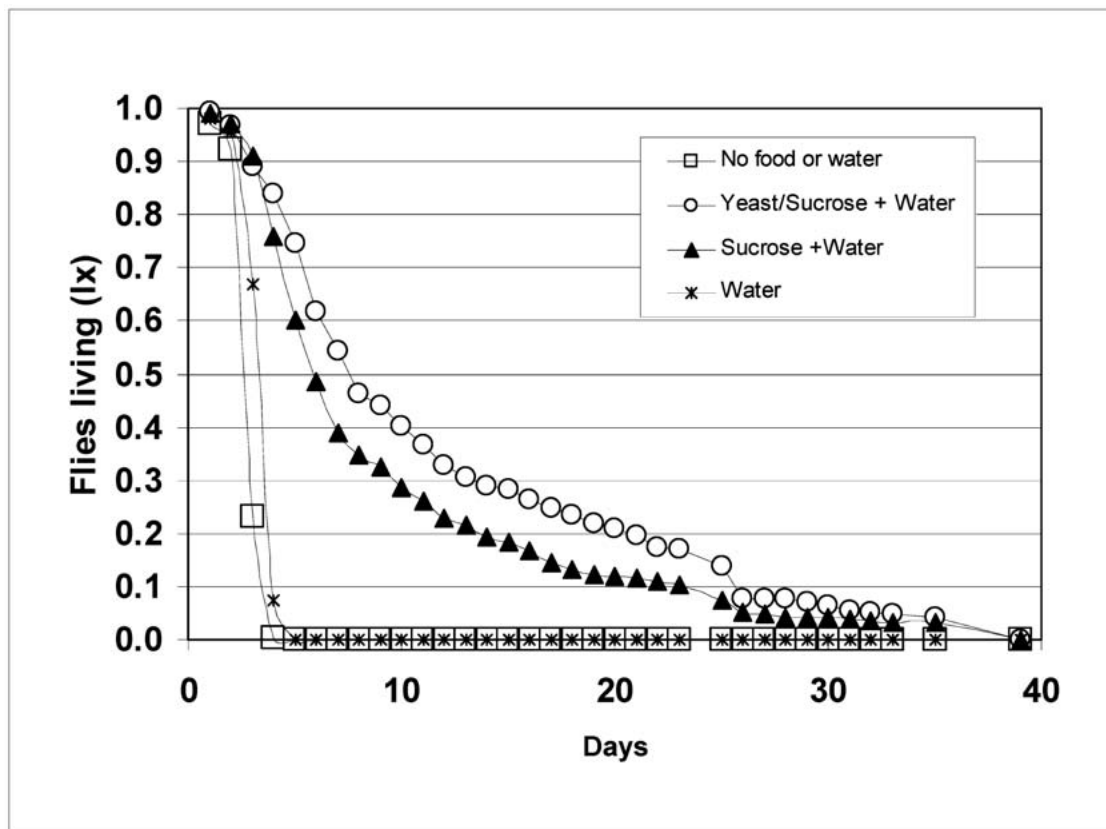


Fig. 1. Adult, sterile male medfly survivorship with different post-release diets offered to flies after 48 h. Results were pooled for both pre-release diets (yeast hydrolysate + sugar and sugar) that were offered to flies for the first 48 h. Day 0 was the day flies were first exposed to post-release diets.

Protein Analysis

Hydrolyzed yeast contained extremely low levels of protein (mean = 3.15 μg protein/mg hydrolyzed yeast, SD = 0.28, $n = 7$). Therefore, the medfly diet that consisted of hydrolyzed yeast + dry sucrose (1:3, wt:wt) was approximately 0.08% protein.

DISCUSSION

The inclusion of a hydrolyzed yeast and sucrose mixture in the post-release adult medfly diet increased longevity in comparison to flies provided with only sucrose and water. There was no increase in the longevity of medflies provided with hydrolyzed yeast and sucrose mixture prior to transfer into cages (i.e., as a pre-release diet) when post-release diets were not provided. This finding leads us to suggest that incorporating this mixture of hydrolyzed yeast and sucrose into the standard pre-release diet of sterile flies would not be beneficial, unless there were other advantages (i.e., improved mating success) that would not be conveyed to these sterile flies if they foraged and

obtained protein in the field. As Maor et al. (2004) stated, released flies incapable of finding protein and carbohydrates are likely to die regardless of the pre-release diet. The length of time a fly is exposed to pre-release conditions (i.e., 2-3 d) may preclude detectable differences in subsequent fly longevity in the field.

The impact of pre-release diets on mating success may be less important than what food sources foraging flies are able to find following release. Kaspi and Yuval (2000) found that mating success of flies offered a protein or sugar pre-release diet for 4 d could be altered by 24 h of starvation (with access to water only) and by offering an apple. For example, sugar-fed males that were given 24 h access to an apple had greater mating success than protein-fed flies starved for 24 h. Likewise, sugar-fed and protein-fed flies both provided with access to apples had comparable mating success. These findings demonstrate that the fate of pre-release flies could vary considerably, largely based on what flies find after they are released.

Mass-reared flies may not die more quickly when fed a diet containing hydrolyzed yeast be-

cause nitrogen does not limit attainment of reproductive maturity (Zucoloto 1992). Thus, because mass-reared flies are fed a larval diet high in protein, adult diet is less important for reproductive maturity than is the case with wild flies. Muller et al. (1997) found that mortality decreased when mass-reared adult medflies were fed a diet containing hydrolyzed yeast. We similarly found a decrease in mortality when cage-confined medfly males were fed a post-teneral diet containing hydrolyzed yeast. Additional studies would be beneficial to continue to tease apart the differences between mass-reared, sterile medflies, and wild medflies in relation to diet.

Surprisingly, we found that hydrolyzed yeast is not a significant source of protein, despite numerous studies that refer to a fly diet containing hydrolyzed yeast or yeast hydrolysate as containing protein or being an important source of protein (Jacome et al. 1995; Muller et al. 1997; Shelly et al. 2002; Yuval et al. 2002). It has been found that yeast hydrolysate contains 19 amino acids and provides an extrinsic source of nitrogen that is needed by walnut husk flies to produce eggs (Tsiropoulos 1978). We were initially concerned that handling of fly diet containing hydrolyzed yeast (e.g., exposure to heat and/or to feeding by flies for extended periods of time), as might occur with cage studies or with storage for use in large-scale SIT programs, could alter protein content. But we found that the protein content was so low to begin with that it is not likely to be affected measurably by adverse conditions. We note that the tephritid fly literature is rife with confusing references to "protein diets" when these diets, for the most part, appear to be "nitrogen-rich" and have contained hydrolyzed yeast, not protein. In the future, researchers should be careful to indicate the identity and relative ratio of components in diets fed to flies.

Based on fly longevity in small field cages (without plants), we did not find overwhelming evidence to suggest that the inclusion of hydrolyzed yeast in SIT pre-release diets is warranted with medfly. Based on our studies and the findings of others, we conclude that the ability of sterile flies to locate and feed on nitrogen and carbohydrate sources post-release will more significantly affect sterile fly survivorship than pre-release diet.

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REFERENCES CITED

- BARRY, J. D., R. V. DOWELL, AND J. G. MORSE. 2002. Comparison of two sterile Mediterranean fruit fly (Diptera: Tephritidae) strains released in California's preventative release program. *J. Econ. Entomol.* 95: 936-944.
- BRADFORD, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principles of protein-dye binding. *Anal. Biochem.* 72: 248-254.
- CANGUSSU, J. A., AND F. S. ZUCOLOTO. 1997. Effect of protein sources on fecundity, food acceptance, and sexual choice by *Ceratitis capitata* (Diptera, Tephritidae). *Rev. Bras. Biol.* 57: 611-618.
- CAYOL, J. 2000. Changes in sexual behavior and life history traits of Tephritid species caused by mass-rearing processes, pp. 843-860 *In* M. Aluja and A. L. Norrbom [eds.], *Fruit flies (Tephritidae): Phylogeny and Evolution of Behavior*. CRC Press, Boca Raton, FL.
- HENDRICH, J., AND M. A. HENDRICH. 1990. Mediterranean fruit fly (Diptera: Tephritidae) in nature: Location and diel pattern of feeding and other activities on fruiting and nonfruiting hosts and nonhosts. *Ann. Entomol. Soc. Am.* 83: 632-641.
- HENDRICH, J., B. I. KATSOYANNOS, D. R. PAPA, AND R. J. PROKOPY. 1991. Sex differences in movement between natural feeding and mating sites and tradeoffs between food consumption, mating success and predator evasion in Mediterranean fruit flies (Diptera: Tephritidae). *Oecologia (Berlin)* 86: 223-231.
- JACOME, I., M. ALUJA, P. LIEDO, AND D. NESTEL. 1995. The influence of adult diet and age on lipid reserves in the tropical fruit fly *Anastrepha serpentina* (Diptera: Tephritidae). *J. Insect. Physiol.* 41: 1079-1086.
- KASPI, R., AND B. YUVAL. 2000. Post-teneral protein feeding improves sexual competitiveness but reduces longevity of mass-reared sterile male Mediterranean fruit flies (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 93: 949-955.
- KASPI, R., P. W. TAYLOR, AND B. YUVAL. 2000. Diet and size influence sexual advertisement and copulatory success of males in Mediterranean fruit fly leks. *Ecol. Entomol.* 25: 279-284.
- KNIPLING, E. F. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. *J. Econ. Entomol.* 48: 459-462.
- LIQUIDO, N. J., L. A. SHINODA, AND R. T. CUNNINGHAM. 1991. Host plants of the Mediterranean fruit fly (Diptera: Tephritidae): an annotated world review. *Misc. Publ. Entomol. Soc. Am.* 77: V-52.
- MAOR, M., B. KAMENSKY, S. SHLOUSH, AND B. YUVAL. 2004. Effects of post-teneral diet on foraging success of sterile male Mediterranean fruit flies. *Entomol. Exp. Appl.* 110: 225-230.
- METCALF, R. L. 1994. Biography of the Medfly, pp. 43-48 *In* J. G. Morse, R. L. Metcalf, J. R. Carey, and R. V. Dowell [eds.], *The Medfly In California: Defining Critical Research*, Center for Exotic Pest Research, University of California, Riverside.
- MINITAB, INC. 1998. MINITAB User's Manual. Minitab, Inc. State College, PA.
- MULLER, H. G., J. L. WANG, W. B. CAPRA, P. LIEDO, AND 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. USA* 94: 2762-2765.

- NIYAZI, N., C. R. LAUZON, AND T. E. SHELLY. 2004. Effect of probiotic adult diets on fitness components of sterile male Mediterranean fruit flies (Diptera: Tephritidae) under laboratory and field cage conditions. *J. Econ. Entomol.* 97: 1570-1580.
- PAPADOPOULOS, N. T., B. I. KATSOYANNOS, N. A. KOULOSSIS, A. P. ECONOMOPOULOS, AND J. R. CARREY. 1998. Effect of adult age, food, and time of day on sexual calling incidence of wild and mass-reared *Ceratitidis capitata* males. *Entomol. Exp. Appl.* 89: 175-182.
- ROBINSON, A. S., U. CIRIO, G. H. S. HOOPER, AND M. CAPPARELLA. 1986. Field cage studies with a genetic sexing strain in the Mediterranean fruit fly, *Ceratitidis capitata*. *Entomol. Exp. Appl.* 41: 231-236.
- SHELLY, T. E., AND S. KENNELLY. 2002. Influence of male diet on male mating success and longevity and female remating in the Mediterranean fruit fly (Diptera: Tephritidae) under laboratory conditions. *Florida Entomol.* 85: 572-579.
- SHELLY, T. E., AND 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. *Florida Entomol.* 86: 340-344.
- SHELLY, T. E., S. S. KENNELLY, AND D. O. MCINNIS. 2002. Effect of adult diet on signaling activity, mate attraction, and mating success in male Mediterranean fruit flies (Diptera: Tephritidae). *Florida Entomol.* 85: 150-155.
- TAYLOR, P. W., AND B. YUVAL. 1999. Postcopulatory sexual selection in Mediterranean fruit flies: advantages for large and protein-fed males. *Anim. Behav.* 58: 247-254.
- TSIROPOULOS, G. J. 1978. Holidic diets and nutritional requirements for survival and reproduction of the adult walnut husk fly. *J. Insect Physiol.* 24: 239-242.
- YUVAL, B., R. KASPI, S. SHLOUSH, AND M. S. WARBURG. 1998. Nutritional reserves regulate male participation in Mediterranean fruit fly leks. *Ecol. Entomol.* 23: 211-215.
- YUVAL, B., R. KASPI, S. A. FIELD, S. BLAY, AND P. TAYLOR. 2002. Effects of post-tenal nutrition on reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). *Florida Entomol.* 85: 165-170.
- YUVAL, B., M. MAOR, K. LEVY, R. KASPI, P. TAYLOR, AND T. SHELLY. 2007. Breakfast of champions or kiss of death? Survival and sexual performance of protein-fed, sterile Mediterranean fruit flies (Diptera: Tephritidae). *Florida Entomol.* 90: 115-122.
- ZUCOLOTO, F. S. 1992. Egg production by *Ceratitidis capitata* (Diptera, Tephritidae) fed with different carbohydrates. *Rev. Bras. Biol.* 36: 235-240.