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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

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ABSTRACT

Using field-caged host trees, this study investigated the influence of adult diet on the mating success and survival of male Mediterranean fruit flies, *Ceratitis capitata* (Wied.), from two mass-rearing strains. Upon emergence, males from a genetic sexing (temperature sensitive lethal) strain and a bisexual strain were given either a sugar-protein diet (protein-fed) or a sugar-only diet (protein-deprived). Mating trials were conducted using field-caged host trees, and 100 males of a given strain and diet competed with 100 wild males for matings with 100 wild females (all wild flies were given the sugar-protein diet). There was no apparent effect of diet on male mating success for either mass-reared strain. Wild males obtained significantly more matings per replicate than males from either strain on either diet, and the mean number of matings per replicate was similar between protein-fed and protein-deprived males for both strains. In addition, the survival of protein-fed and protein-deprived males was compared over 2- and 4-day intervals on field-caged host trees. Within each mass-reared strain, there was no apparent effect of adult diet on male survival for either test interval. The present findings are compared with similar studies on other mass-reared strains.

Key Words: *Ceratitis capitata*, nutrition, copulation, sterile insect technique

RESUMEN

Utilizando árboles hospederos dentro de jaulas de campo, este estudio investigó la influencia de la dieta del adulto en el éxito de apareamiento y la sobrevivencia de los machos de la mosca mediterránea de la fruta, *Ceratitis capitata* (Wied.), en dos razas criadas en masa. Después de emerger, los machos de una raza genética sexual (letalmente sensible a la temperatura) y una raza heterosexual alimentados con una dieta de proteína-de azúcar (alimentados con proteína) o una dieta de azúcar solamente (privados de proteína). Pruebas de apareamiento fueron conducidos usando árboles hospederos dentro de jaulas de campo, y 100 machos de una cierta raza y dieta competieron con 100 machos silvestres para aparearse con 100 hembras silvestres (todas las moscas silvestres fueron alimentadas con una dieta de proteína-azúcar). No hubo un efecto aparente de la dieta sobre el éxito de los machos para aparearse con cualquiera de las razas criadas en masa. Los machos silvestres aparearon significativamente más veces por réplica que los machos de ambas razas en cualquiera de las dietas, y el número promedio de los apareamientos por replica fué similar entre los machos alimentados con proteína y los privados de proteína en las dos razas. Además, se comparó la sobrevivencia de los machos alimentados con proteína, con privados de proteína en un intervalo de 2 y 4 días en árboles hospederos dentro de jaulas de campo. Entre cada raza criada en masa, no había un efecto aparente de la dieta del adulto sobre el sobrevivencia de machos en cualquiera de intervalos de prueba. Se comparan estos descubrimientos con estudios similares en razas criadas en masa.

In a series of recent articles, Yuval and his colleagues have demonstrated the importance of adult diet, specifically the inclusion of protein (yeast hydrolysate), on the mating success and longevity of male Mediterranean fruit flies, *Ceratitis capitata* (Wied.). In the initial study, Blay and Yuval (1997) worked with mass-reared flies and found that, in no-choice tests, females mated more readily with protein-fed males than protein-deprived males and that remating frequency was

lower for females that first mated with protein-fed males than protein-deprived males. Subsequently, Kaspi et al. (2000) observed wild flies on field-caged host trees and found that protein-fed males signaled (pheromone-called) and mated more frequently than protein-deprived males. In another laboratory study, Kaspi and Yuval (2000) investigated the influence of adult diet on mating competition among mass-reared males for wild females and found that protein-fed males had a

mating advantage over protein-deprived males. However, protein-fed males were found to have lower longevity than protein-deprived males.

These findings potentially have great importance for sterile male release programs against the Mediterranean fruit fly. While the pre-release diet in current use is a sugar agar gel, the above studies suggest that the addition of protein may significantly enhance the mating competitiveness of mass-reared males and hence the effectiveness of sterile release programs. Before widespread adoption of this dietary change, however, data on other pairwise combinations of mass-reared and wild strains should be gathered to determine the generality and strength of protein-mediated effects on the mating success and longevity of male Mediterranean fruit flies.

The present study investigates the effect of dietary protein on the mating success and longevity of Mediterranean fruit fly males from two mass-reared strains (Vienna-7/Tol-99 and Maui Med-93) in field-caged host trees in Hawaii. This research expands upon an earlier project (Shelly & Kennelly 2002) in which the mating success and longevity of males from one of these strains (Maui Med-93) were measured under laboratory conditions. This earlier study showed that, in trials with wild flies, protein-fed males had a significant mating advantage over protein-deprived males. However, the addition of protein to the diet did not boost the mating success of mass-reared males in competition with wild males or mass-reared males for wild females. In addition, no difference was found in the survival probability of protein-fed versus protein-deprived males from the mass-reared strain. The present study was undertaken 1) to verify these trends under more natural conditions (field-cages) and 2) to gather comparable data for males from a genetic sexing strain (Vienna-7/Tol-99) used widely (e.g., in California and Guatemala) in ongoing sterile insect release programs.

MATERIALS AND METHODS

Study Insects

Wild flies were reared from infested coffee, *Coffea arabica* L., berries collected on the island of Kauai. Fruits were held over vermiculite at 23-25°C, and larval development proceeded *in situ*. Puparia were sifted from the vermiculite 7-9 days after fruit collection, and adults were separated by sex within 2 days of eclosion, well before reaching sexual maturity at 7-10 days of age. Adults were held in plastic buckets covered with nylon screening (volume 5 liters; 100-125 flies per bucket). Wild flies were provided with a mixture (3:1 v/v) of sugar (sucrose) and protein (yeast hydrolysate) and water *ad libitum*, held at 20-24°C and 65-85% RH, and received both natural and artificial light in a 12:12 (L:D) photoperiod.

Sterile males from two mass-reared strains were used in the mating and survival trials. One was a temperature sensitive lethal (or *tsl*) strain (Vienna-7/Tol-99, hereafter referred to as Vienna-7), a type of genetic sexing strain in which females are selectively killed in the egg stage by exposure to high temperature (Franz et al. 1994), obtained from the California Department of Food and Agriculture Fruit Fly Rearing Facility, Waimanalo, Oahu. The other was a bisexual strain (Maui Med-93) produced by the USDA-APHIS Hawaii Fruit Fly Rearing Facility, Waimanalo. Larvae of both strains were reared on standard larval medium (Tanaka et al. 1969), and males from both strains received an absorbed dose of 150 Gy of gamma radiation from a ¹³⁷Cs source 2 days before emergence and then delivered to the laboratory. For the Maui Med-93 strain, males were collected within 12 h of emergence (males of this strain become sexually mature at 2-4 days of age). Mass-reared males were separated into 2 dietary regimes: "protein-fed" males were given the same sugar-protein mixture as wild flies plus water, and "protein-deprived" males were given only sugar plus water. Aside from this dietary difference, mass-reared males were maintained in the same manner as wild flies.

Mating Trials

Mating tests were conducted at the Agricultural Experiment Station of the University of Hawaii, Waimanalo. Groups of 100 irradiated, mass-reared males (from the same strain and diet), 100 wild males (10-15 days old), and 100 wild females (10-17 days old) were released between 0800-0830 h in field-cages (2.5 m in height, 3.0 m in diameter) that contained a single rooted guava, *Psidium guajava* L., tree. For a given trial, we marked only males from one group (i.e., mass-reared or wild) and alternated the identity of the marked group between successive trials. Males were marked 1 day before testing by cooling them for several minutes and placing a dot of enamel paint on the thorax. This procedure had no obvious adverse effects, and males resumed normal activities within minutes of handling. The cages were monitored for 4 h, mating pairs were collected in vials, and the males were identified. For each diet, 11 replicates were run using males from the Vienna-7 strain, and 8 replicates were run using Maui Med-93 males. New flies were used in all replicates.

Survival

Two experiments were conducted to examine the effect of adult diet on survival of males from both mass-reared strains. In both cases, 100 protein-fed and 100 protein-deprived males were released into field-cages when 4 days old (the approximate age of release in control programs).

In the first experiment, no food or water was placed in the field-cages (the guava trees contained no fruits or flowers), and survivors were collected and counted 2 days later. In the second, food and water were placed in the field-cage, and survivors were collected and counted 4 days later. Slices of papaya, *Carica papaya* (L.), and orange, *Citrus sinensis* (L.), fruits were presented in an open-sided, covered container suspended in the canopy, and a water container (500 ml) with an emergent cotton wick was wedged in a branch fork. For a given trial, we marked only males from one group (i.e., protein-fed or protein-deprived) and alternated the identity of the marked group between successive trials. Marking procedures followed those described above. For both mass-rearing strains, the survival experiments were run using the same two caged guava trees, and 12 replicates (6 per tree) were performed for the 2-day test, and 16 replicates (8 per tree) were performed for the 4-day test.

Statistical Analysis

Comparisons of mating success and survivorship were made using the t test as assumptions of normality and homoscedascity were met in all cases. Tests were performed using SigmaStat Statistical Software (Version 2.0). Means (± 1 SD) are presented.

RESULTS

Diet had no apparent effect on the mating success of males from either the Vienna-7 or Maui Med strains. Wild males obtained significantly more matings per replicate than mass-reared males from either strain held on either diet (Table 1). Also, the mean number of matings per replicate did not differ significantly between protein-

fed and protein-deprived males for either the Vienna-7 ($t = 0.7$; $df = 20$; $P = 0.48$) or Maui Med ($t = 0.2$; $df = 20$; $P = 0.61$) strain. Pooling data across diets, we found no difference in relative mating success between the two mass-reared strains: Vienna-7 and Maui Med-93 males obtained 30.1% and 31.5% of the total matings per replicate, respectively, (proportions were arcsine transformed for analysis; $t = 0.3$; $df = 42$; $P = 0.67$).

Likewise, diet had no detectable effect on survival of males from either the Vienna-7 or Maui Med strains. The mean number of survivors did not differ between the 2 trees used for either diet treatment for either strain (t test; $P > 0.05$ in all cases); consequently, data were pooled across the trees. The number of protein-fed and protein-deprived males surviving did not differ, on average, for either mass-reared strain in either the 2-day or the 4-day tests (Table 2). Independent of diet, there was a significant difference in the number of survivors between the Vienna-7 ($x = 61.5 \pm 16.4$) and Maui Med-93 ($x = 39.6 \pm 11.5$) males in the 2-day test ($t = 5.4$, $df = 46$. $P < 0.001$). A similar trend was observed in the 4-day test (Vienna-7: $x = 42.5 \pm 8.9$; Maui Med-03: $x = 36.9 \pm 8.0$), but in this case the difference was not statistically significant ($t = 1.1$; $df = 62$; $P = 0.26$).

DISCUSSION

The present findings revealed that, in direct competition with wild males for wild females, the addition of protein (yeast hydrolysate) to the adult diet had no detectable effect on the mating success of males from two mass-reared strains. Wild males obtained the majority of matings in all experiments, and, independent of diet, males from both the Vienna-7 and Maui Med-93 strains obtained only about 30% of the total matings. In addition, diet had no apparent effect on the survival of mass-reared males. Within each strain, survival probability was similar between protein-fed and protein-deprived males in both the 2- and 4-day tests. Independent of diet, male survival differed significantly between the strains in the 2-day test, with males from the Vienna-7 having a higher survival probability than males of the Maui Med strain. However, male survival was similar in the 2 strains over a 4-day interval.

As noted above, data on the importance of dietary protein are equivocal. To summarize, the addition of protein to the adult diet has been found to enhance mating success in 1) all cases involving competition between wild males (Kaspi et al. 2000; Shelly et al. 2002; Shelly & Kennelly 2002), 2) only some cases involving competition among mass-reared males (Kaspi & Yuval 2000; Shelly & Kennelly 2002), and 3) no instances involving mass-reared males competing against wild males (Shelly & Kennelly 2002; Shelly et al. 2003; this study). It is not known why dietary pro-

TABLE 1. MATING SUCCESS OF PROTEIN-FED AND PROTEIN-DEPRIVED MASS-REARED MALES IN DIRECT COMPETITION WITH WILD MALES FOR COPULATIONS WITH WILD FEMALES. VALUES REPRESENT MEAN NUMBER OF MATINGS (± 1 SD) PER REPLICATE (N = 11 FOR VIENNA-7 AND 8 FOR MAUI MED-93).

Male type	Matings	t
Wild	18.7 (3.4)	8.6***
Vienna-7: Protein-fed	7.2 (2.8)	
Wild	17.3 (4.0)	5.2***
Vienna-7: Protein-deprived	8.3 (4.2)	
Wild	19.2 (4.1)	5.6***
Maui Med-93: Protein-fed	9.1 (3.8)	
Wild	20.1 (4.6)	5.5***
Maui Med-93: Protein-deprived	9.0 (4.4)	

***P < 0.001.

TABLE 2. SURVIVORSHIP OF PROTEIN-FED VERSUS PROTEIN-DEPRIVED MASS-REARED MALES. VALUES REPRESENT MEAN NUMBER (± 1 SD) OF SURVIVORS (OF 100 INDIVIDUALS RELEASED) PER REPLICATE EITHER 2 (NO FOOD AND WATER ADDED) OR 4 (FOOD AND WATER ADDED) DAYS AFTER RELEASE IN FIELD-CAGES. FOR EACH STRAIN, DATA WERE POOLED OVER THE 2 TREES USED, AND N = 12 REPLICATES FOR THE 2-DAY TEST AND N = 16 REPLICATES FOR THE 4-DAY TEST.

Strain	Interval	Diet	Survivors	t
Vienna-7	2 days	Protein-fed	61.4 (15.8)	0.03 ^{NS}
		Protein-deprived	61.7 (19.8)	
	4 days	Protein-fed	41.8 (7.5)	0.43 ^{NS}
		Protein-deprived	43.3 (10.5)	
Maui Med-93	2 days	Protein-fed	45.6 (12.3)	1.44 ^{NS}
		Protein-deprived	38.2 (12.2)	
	4 days	Protein-fed	36.5 (9.9)	0.40 ^{NS}
		Protein-deprived	37.2 (7.1)	

^{NS}P > 0.05.

tein has a consistently positive effect on mating success for wild males but not for mass-reared males. C. Lauzon (pers. comm.) suggested that, because they developed on fruit without subsequent exposure to irradiation, wild males possessed a natural gut flora whose growth was greatly stimulated by protein. This proliferation, in turn, may have heightened metabolic processes that increased energy stores available for sexual activities. Available data on this point are inconsistent. Kaspi et al. (2000) found that protein-fed wild males signaled more frequently than protein-deprived wild males, whereas Shelly et al. (2002) found no influence of diet on calling activity. A natural community of gut microbes may have also facilitated synthesis of compounds important in sexual activities (e.g., pheromone precursors). Little data exist on potential dietary influences on long-range, female attraction, but Shelly et al. (2002) found no difference in female visitation between protein-fed versus protein-deprived males in a field-cage test. In contrast to wild males, mass-reared, irradiated males are more likely to lack a natural, microbial community. Consequently, dietary protein may not stimulate key metabolic processes as dramatically as in wild males, and sterile males show no or variable increase in mating frequency as a result.

Data on the effects of dietary protein on male survival are scant and conflicting. In the laboratory, Kaspi and Yuval (2000) found that, after 4 days of feeding, protein-fed males were less likely to survive a 24 h period of starvation (on day 5) than protein-deprived males, whereas Shelly & Kennelly (2002) found that male survival varied independently of diet both when food was available continuously or removed after several days. Here, we found no effect of diet on the survival of mass-reared males in field-caged trees. While a more natural situation, the caged trees, although lacking fruits, flowers, or bird feces, may have

harbored microbial populations as well as insect honeydew and frass on leaf and bark surfaces. These potential food sources would, of course, be available to all males and would therefore act to reduce any nutritional differences that existed between protein-fed and protein-deprived males prior to their release on the tree.

Independent of dietary considerations, the present study once again highlights the need to develop and implement rearing procedures that improve the mating performance of mass-reared males for control of the Mediterranean fruit fly. Similar to other studies (reviewed by Cayol 2000), our results show that, relative to wild males, mass-reared males are poor competitors in the sexual arena. Echoing earlier advice (Calkins 1984), we therefore urge the development of rearing procedures that attempt to increase the quality as well as the quantity of sterile Mediterranean fruit flies produced.

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