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DIETARY PROTEIN AND MATING COMPETITIVENESS OF STERILE MALES OF THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE): MEASUREMENTS OF INDUCED EGG STERILITY IN LARGE FIELD ENCLOSURES

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The Sterile Insect Technique (SIT) is widely used to control infestations of the Mediterranean fruit fly (or medfly), *Ceratitis capitata* (Wied.) (Hendrichs et al. 2002). There is, however, considerable evidence (Lance et al. 2000) showing that mass-reared, sterile males are inferior to wild males in mating competition for wild females. Blay and Yuval (1997) demonstrated that the addition of protein (yeast hydrolysate) to the standard sugar-agar diet increased the mating competitiveness of sterile males. However, research conducted in Hawaii failed to detect a similar protein effect (Shelly & McInnis 2003). Thus, the potential role of dietary protein in improving the effectiveness of medfly SIT remains uncertain.

This study compares levels of egg sterility in large field enclosures containing fertile flies and protein-fed or protein-deprived sterile males. In the aforementioned studies, mating trials were run over short intervals in laboratory cages or on single host trees and thus precluded potential effects of habitat heterogeneity and inter-tree movement on male mating competition. In addition, data on mating success do not necessarily mirror the level of egg sterility realized in the open field, because they ignore the possibility that females mate multiple times, even on the same day (Vera et al. 2003), and consequently that sperm competition may be occurring. While not a complete substitute for a long-term, open-field test, the protocol described below involved measurements of egg sterility over several days in enclosures holding multiple host trees, and the resulting data were presumably more reflective of natural conditions.

The flies used in this study were maintained following the protocol of Shelly et al. (2005). Owing to the limited availability of wild flies, we used flies from a recently established colony (REC) reared from field-collected coffee berries. Larvae were reared on artificial media, and adults were separated within 24 h of emergence and maintained on a sugar-protein mixture and water. When used, REC flies were 7-13 d old and 4-7 generations removed from the wild. Mass-reared flies were from a *tsl* strain (Vienna-7/Tol-99) produced by the California Department of Food and Agriculture Hawaii Fruit Fly Rearing Facility, Waimanalo, Oahu. Two d before eclosion, pupae were coated with fluorescent dye (standard marking procedure), irradiated, and placed in storage (so-called PARC) boxes. For a given trial, we placed 85 ml of pupae (60 pupae per ml) in each of 2 boxes, which yielded ≈4,080 flying adults (≈80% of *tsl* male pupae yield adults capable of flight, California Department of Agriculture, unpubl.). On the day of peak emergence, males in one box were provided with a slab of sugar-agar gel (standard size and formulation placed on the screened panel on the box lid), while males in the other box were provided with a sugar-agar slab plus the sugar-protein mixture. Boxes receiving this mixture had a hole drilled in one side through which we introduced 2 Petri dishes of the sugar-protein food (some mixture was invariably present on the day of male release, indicating that the supply of protein-containing food was not limited). The *tsl* males were held in the boxes for 4 d after peak emergence (i.e., 6 d after pupal placement) and then released in the field enclosures.

The methods and schedule of egg collection followed Shelly et al. (2005). Trials were conducted in 2 nylon-screened enclosures (16 by 6 by 2.5 m, l:w:h) erected in Waimanalo, Oahu, that contained 10 and 12 guava trees (*Psidium guajava* L.), respectively. Protein-deprived and protein-fed males were tested concurrently (1 treatment per enclosure), and treatments were alternated between the 2 enclosures in successive replicates. Trials were conducted during November 2004-March 2005, with daily maximum temperatures ranging between 23-28°C.

On d 1 of a replicate, we released 200 REC males, 200 REC females, and ≈4,080 *tsl* males of a given diet type (i.e., a ratio of ≈20:1, sterile:wild males) in the center of each enclosure between 0900-0930 h (males were released 20 min before females). Also, on d 1 food (sugar-agar) and water were placed at 4 locations in the enclosures and replaced daily. On d 2, 12 Granny Smith apples (*Malus domestica* Borkh.) were placed in each enclosure at 1000 h for oviposition (guava fruits were removed before the trials). Apples were suspended 1.5-2.5 m above ground by piercing the fruit with a nail and connecting the nail to a branch with wire. On d 3 and 4 at 1000 h, apples were collected and replaced with new ones. On d 5, apples were collected but not replaced, marking the end of the trial. Collected apples were returned to the laboratory, and eggs were removed with a scalpel and forceps. Eggs were placed on moistened blotter paper within Petri dishes, incubated at 27°C for 48 h, and then scored for hatching. Eight replicates

were conducted per diet regime, with successive trials separated by 7 d. Between trials, surviving flies were eradicated through trapping (trimedlure and food baits) and visual searching.

For each replicate (i.e., concurrent pair of trials), we also measured egg hatch of REC females mated exclusively to REC males in a smaller field-cage (3.0 m diameter, 2.5 m high) over a single guava tree adjacent to the large enclosures. One hundred individuals of each sex were introduced on d 1, and 2 apples were introduced on d 2 for 24 h. These data were used in the computation of Fried's (1971) competitiveness index (C; egg hatch in REC female by sterile male matings was assumed to be zero based on data from Shelly et al. (2005)).

There were no significant differences between diet treatments in the number of eggs collected for a given day or for an entire replicate (t test, $P > 0.05$ in all cases, Table 1). For both diets, however, the number of eggs collected differed significantly among days, with a steady decrease noted over time (Kruskal-Wallis test, $P < 0.05$ in both cases). There was no significant difference between diet treatments in the proportion of unhatched eggs collected for a given day or for an entire replicate (t test after arc sine transformed percentages, $P > 0.05$ in all cases, Table 1). Also, unlike egg number, the proportion of unhatched eggs varied independently of day for both diet treatments (ANOVA with arc sine transformed percentages, $P > 0.05$ in both cases).

In the field-cage containing REC flies only, 319.6 eggs (SE = 32.9) were collected per replicate of which 21% (SE = 2.6), on average, did not hatch. C values were computed for individual replicates for each diet treatment, and overall mean values of C did not differ between protein-deprived (mean = 0.27, SE = 0.07) and protein-fed (mean = 0.19, SE = 0.04) males (t test, $P > 0.05$).

TABLE 1. NUMBER OF EGGS COLLECTED AND PROPORTION OF UNHATCHED EGGS PER DAY AND PER REPLICATE FOR FIELD ENCLOSURES CONTAINING FERTILE FLIES AND STERILE *tsl* MALES FED EITHER SUGAR-AGAR ONLY OR SUGAR-AGAR PLUS A SUGAR-PROTEIN MIXTURE. VALUES REPRESENT MEANS WITH STANDARD ERROR (SE) OF 8 REPLICATES.

Sterile male diet	Day[1]	No. eggs collected	% Unhatched eggs
Sugar only	2	640.6 (165.8)	85.3 (2.4)
	3	192.2 (41.0)	79.9 (5.4)
	4	100.6 (14.5)	85.9 (3.4)
	Total	933.5 (192.2)	84.3 (2.5)
Sugar + Protein	2	848.8 (170.3)	77.9 (4.1)
	3	296.2 (58.2)	80.2 (4.0)
	4	141.6 (25.7)	82.2 (4.6)
	Total	1286.7 (219.4)	79.0 (3.7)

[1]Day that groups of 12 apples were placed in enclosures; flies were released on d 1.

Our results show no effect of dietary protein on the level of egg sterility induced by sterile males. Interpretation is potentially confounded by the likelihood that the sterile males ingested feces deposited on the box walls (as reported by Blay & Yuval 1997). Any supplementary nutrients so obtained by the protein-deprived males, in particular, might have acted to reduce behavioral differences arising from the experimental dietary treatments. This explanation is, of course, more important from a physiological perspective than an operational one, since it is the outcome (no diet-related difference in induced egg sterility), and not the mechanism, that is most relevant to program managers in medfly SIT. In addition, results should be interpreted with caution, because recently colonized flies were used in lieu of wild flies.

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SUMMARY

Competitive mating environments were established in large field enclosures by placing fertile medflies with protein-deprived or protein-fed sterile males. Based on the hatch rate of eggs collected from fruits over several days, the addition of protein to the adult diet had no effect on the mating competitiveness of sterile males.

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