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(DIPTERA: TEPHRITIDAE)**

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## BREAKFAST OF CHAMPIONS OR KISS OF DEATH? SURVIVAL AND SEXUAL PERFORMANCE OF PROTEIN-FED, STERILE MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHTRITIDAE)

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### ABSTRACT

The sterile insect technique (SIT) is increasingly being used around the world to control *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), the Mediterranean fruit fly as part of an area-wide integrated approach. One option that may improve the effectiveness of the SIT, by increasing the sexual competitiveness of released sterile males, consists of feeding males protein during the post-teneral stage, a diet that increases sexual performance of wild males. We examine the effects of diet on the successive hurdles males must overcome in order to inseminate females, i.e., joining leks, copulating females, having their sperm stored and inhibition of female remating. In addition, we address the effects of diet on post-release foraging success, longevity, and the ability to withstand starvation. While protein feeding universally increases the sexual success of wild males, its effect on sterile males varies with strain, experimental settings, and environmental conditions. In some cases, treatments that resulted in the best sexual performance were significantly associated with increased vulnerability to starvation. However, no particular diet affected the ability of sterile males to find nutrients in the field when these were available. We suggest it may be better to release relatively short-lived flies that are highly competitive, rather than long-lived, sexually ineffective ones.

**Key Words:** Tephritidae, *Ceratitis capitata*, Sterile Insect Technique, nutrition, sexual behavior, longevity, starvation

### RESUMEN

El uso de la técnica de insecto estéril (TIE) esta aumentando alrededor del mundo para el control de *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), la mosca mediterránea de la fruta como parte de un enfoque integrado por toda el área. Una opción que puede mejorar la eficiencia de TIE, por medio del aumento de la capacidad de los machos esteriles liberados para competir, consiste en la alimentación de los machos con proteína durante la etapa de pos-teneral, una dieta que aumenta el desempeño sexual de los machos naturales. Nosotros examinamos los efectos de la dieta sobre los obstáculos sucesivos que los machos tienen que superar para inseminar las hembras, i.e., unir con otros machos en áreas para el apareamiento, copula de las hembras, almacenar esperma e inhibir el re-apareamiento de hembras. Además discutimos los efectos de la dieta sobre el éxito de su actividad forrajera después de ser liberados, la longevidad y la habilidad para aguantar la inanición. Mientras que la alimentación con proteína universalmente aumentan el éxito sexual de los machos naturales, su efecto sobre los machos estériles varía según la raza, el lugar de los experimentos y las condiciones ambientales. En algunos casos, los tratamientos que resultaron con mejor desempeño sexual fueron asociados significativamente con el aumento de la vulnerabilidad a la inanición. Sin embargo, ninguna dieta en particular afectó la habilidad de los machos estériles para encontrar nutrientes en el campo cuando fueron disponibles. Nosotros sugerimos que puede ser mejor el liberar moscas que tienen una vida relativamente corta y que son mas competidoras, en vez de moscas que tienen una vida larga y sexualmente inefectiva.

Most of the tephritid fruit flies are anautogenous, i.e., females need to feed on a source of protein to mature eggs, while males need protein for pheromone production, the renewal of sperm supplies, and production of male accessory gland se-

cretions (Drew & Yuval 2000). Male Mediterranean fruit flies (medflies) *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), are no exception. We have documented, for both wild and laboratory reared males, how post-teneral pro-

tein feeding affects their ability to join leks (Yuval et al. 1998), copulate in leks (Kaspi et al. 2000; Shelly et al. 2002), transfer a substantial ejaculate (Taylor & Yuval 1999), and inhibit female remating (Blay & Yuval 1997). When synthesizing and reviewing these findings (Yuval & Hendrichs 2000; Yuval et al. 2002), the importance of post-teneral nutrition for the effective performance of mass-reared, sterile males in the field was suggested.

For the sterile insect technique (SIT) to succeed, it is imperative that released sterile males compete successfully against males from the wild population. This entails joining or establishing leks in the field, attracting and courting wild females, copulating them, and, finally, inhibiting their receptivity to further copulations, all the while competing with wild males. Accordingly, a significant research effort has focused on establishing ways to understand the basis of male competitiveness and enhance that of sterile males (Shelly 1999; Kaspi & Yuval 2000; Shelly & McInnis 2001; Briceño et al. 2002; Robinson et al. 2002). Furthermore, although some released males may be quite long-lived in the field (Plant & Cunningham 1991), their effectiveness in copulating and inseminating females as they age was unknown. If their potency declines significantly, their survivability becomes less crucial for the success of the SIT.

In this review, we examine a number of recent studies that look at the effect of protein diet on sexual performance and survival of sterile, mass reared male medflies in various experimental settings. We examine the effects of diet on the successive hurdles males must overcome in order to inseminate females, namely joining leks, copulating females, transferring sperm, and inhibition of female remating. In addition, we address the effects of diet on male post-release foraging success, longevity, and the ability to withstand starvation. Because the effects of protein diet on sterile male sexual performance and survival are equivocal, we dwell on the experimental conditions of the various studies, the strains used, and environmental effects that may have affected the results.

## SEXUAL PERFORMANCE OF STERILE MALES

### Participation in Leks

Kaspi & Yuval (2000), working in field cages, found that protein-fed sterile males of the VIENNA 4/Tol-94 sexing strain (Robinson et al. 1999), (flown from Guatemala to Israel), were significantly more likely to join leks and emit pheromone (call) than sterile males fed only sugar. This effect was significant for the 2 age groups tested, 4-d-old and 6-8-d-old males. These experiments were performed without females and without competition between males of the 2 diet treatments.

In another experiment with the VIENNA 7/Tol-2000 sexing strain (Franz 2005), participation of protein-fed or protein-deprived (sugar-fed) sterile males in leks on the days following their release into a large field enclosure (8 × 3 square meters, 2.5 meters high) was monitored (Maor et al. 2004). Males emitting pheromone in leks were sampled for 30 min each day after release. Numbers of calling males from both diets declined significantly from day to day, but the post-teneral diet had no significant effect on calling in leks.

Maor (2004) attempted to find an alternative protein-rich diet to the hydrolyzed yeast commonly used, one that would have a significant effect on lek joining in simulated field conditions. Sterile males of the VIENNA 7/Tol-2000 strain were offered one of the following diets after they emerged: (i) sugar; (ii) sugar and protein (hydrolyzed yeast presented in a separate dish to allow optimal self selection (e.g., Cangussu & Zucoloto 1995), (iii) sugar and a protein pulse on d 2, or (iv) a slice of dry, but rehydrated, apricot to mimic a protein rich natural diet. Four d after emergence, flies were released in a field enclosure, and lek participation was monitored for 6 d. A significant temporal decline in lek participation was observed for all diets. Furthermore, the post-teneral diet significantly affected lek participation. The highest level of participation was exhibited by apricot fed males (iv), followed by the sugar (i) and sugar + protein-fed (ii) flies. The flies fed a protein pulse on d 2 (iii) contributed the fewest numbers to the lekking population (Fig. 1).

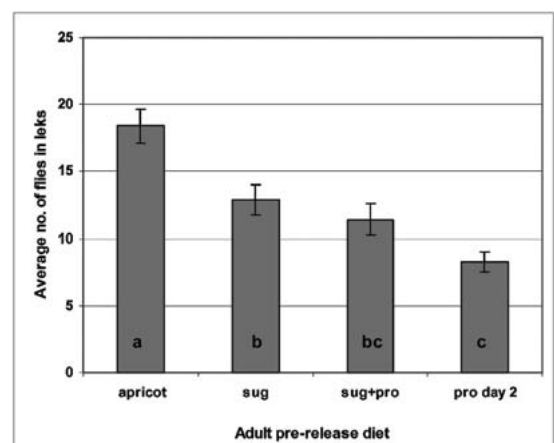


Fig. 1. Effect of pre-release diet on subsequent participation in leks by sterile male Mediterranean fruit flies. Diets were: 1. "apricot"—dry, sliced apricot; 2. "sug"—20% sucrose; 3. "sug + pro"—sucrose plus unlimited hydrolyzed protein; 4. "pro day 2"—unlimited sucrose, protein only on d 2. Bars represent 2 standard errors. Different letters in columns denote a significant difference ( $P < 0.05$ ) in male abundance.

The total amount of protein and lipid in each male was assayed. Diet significantly affected protein levels ( $H = 128$ ,  $df = 4$ ,  $P < 0.0001$ ). On emergence, the males contained on average  $28.47 \pm 1.23$   $\mu\text{g}$  of protein. This increased significantly in all groups at the time of release, with the greatest increase found in males who enjoyed diets containing hydrolyzed yeast (Fig. 2a). Lipid levels were significantly different between diets ( $F = 6.51$ ;  $df = 4, 223$ ;  $P < 0.0001$ ), but show a different pattern (Fig. 2b). The highest levels of lipid, at 4 d of age (day of release), were found in the flies that were fed sugar only or sugar with a pulse of protein.

This experiment demonstrated that the apricot diet, a natural source of protein and carbohydrate, enhanced the sexual performance of the sterile males. This enhancement was not reflected in the total amounts of nutrients present on the day of release (Fig. 2), suggesting either a synergistic effect or the action of qualitative agents present in the dried fruit. We repeated this experiment in field cages on the Hawaiian island of Oahu, using sterile males of the VIENNA 4/Tol-94 strain obtained as pupae from the California Department of Food and Agriculture rearing facility in Hawaii (Maor 2004). In these experiments, despite similar temporal trends in lekking behavior, the apricot diet did not confer a significant advantage to the males who consumed it.

#### Copulatory Success

Kaspi & Yuval (2000) allowed protein and sugar fed sterile males of the VIENNA 4/Tol-94 strain to compete against wild males fed only sugar for copulations with wild females (providing a 2:1 sex ratio). Significantly, protein-fed males were more likely to copulate. This effect was significant for the 2 age groups tested—4-d-old males and 6-8-d-old males. Similarly, Shelly & Kennelly (2002) investigated the effects of dietary protein on the mating competition of wild and mass-reared (Maui-Med bisexual strain from Hawaii) males for copulations with wild females. Importantly, they found that although protein in the adult diet improved the copulatory success of wild males, it did not improve the ability of sterile, mass reared males to compete against wild males for copulations with wild females.

Shelly & McInnis (2003) reconfirmed this finding in a follow up study conducted in field cages. Protein-fed, sterile males of 2 mass reared strains—VIENNA 7/Tol-2000 and Maui-Med bisexual strain—were unable to compete any better against wild males for copulations with wild females than only sugar-fed males.

In another experiment, artificial diets were removed from 4-d-old sterile males, and a natural diet (apple slice) was offered. After 24 h, there was no difference between sugar-fed and protein-fed males in copulatory success with wild females

in a field cage (Kaspi & Yuval 2000). Furthermore, when males were starved instead of offered a natural diet, virtually none copulated. A similar result was obtained by Shelly & Kennelly (2003) when starved wild males were assayed for copulatory success. They found that wild males unable to locate food for 1 d have reduced copulatory success on the following day compared to successful foragers. Males starved for 24 h obtained only half as many matings as fed males; this outcome was independent of the pre-starvation diet. When the starvation period was only 18 h, diet did affect mating performance—protein fed males performed poorly, while sugar fed males mated as often as fed males.

The combined effects of diet and exposure to  $\alpha$ -copaene, a plant derived volatile that enhances male sexual success (Shelly 2001), on copulatory success of sterile males of the VIENNA 7/Tol-2000 strain was examined in field enclosures in Guatemala (Shelly et al. 2003). This study pitted sterile males against wild males and was replicated at 2 elevations, 700 and 1,200 m above sea level. While exposure to ginger root oil (containing  $\alpha$ -copaene) enhanced male performance irrespective of diet or elevation, protein-fed males performed significantly better than sugar-fed males only at the high elevation site. Intriguingly, overall performance of sterile males at the higher elevation was significantly worse than at the lower elevation. Accordingly, this study, while confirming the utility of exposure to  $\alpha$ -copaene, could not provide unambiguous support for the inclusion of protein in the pre-release diet, and indicated that the conditions favoring such a diet need to be established with greater specificity.

#### Post-Copulatory Effects

**Sperm Storage.** Taylor et al. (2001), working with males of the VIENNA 4/Tol-94 strain mating with wild females in field cages, found that the probability of sperm storage decreased significantly with male age but was not significantly affected by male diet. As males aged, there was a significant decline in the number of sperm stored by their mates (median: 1263 sperm; range: 20-5684 sperm), yet number of sperm stored did not vary with male diet, male size, or female size.

**Copula Duration.** Taylor et al. (2000, 2001) showed that copulations of sterile males with wild females culminating in sperm storage lasted a median of 186.5 min, whereas failed copulations (no sperm storage) lasted a median of 157.5 min. Despite the considerable overlap in duration of these copulations, the relationship between copula duration and probability of sperm storage was significant. However, duration of inseminating copulations did not vary with any of the investigated fly qualities (male age, diet, or size of males and females). Similarly, Shelly & Kennelly (2002) found

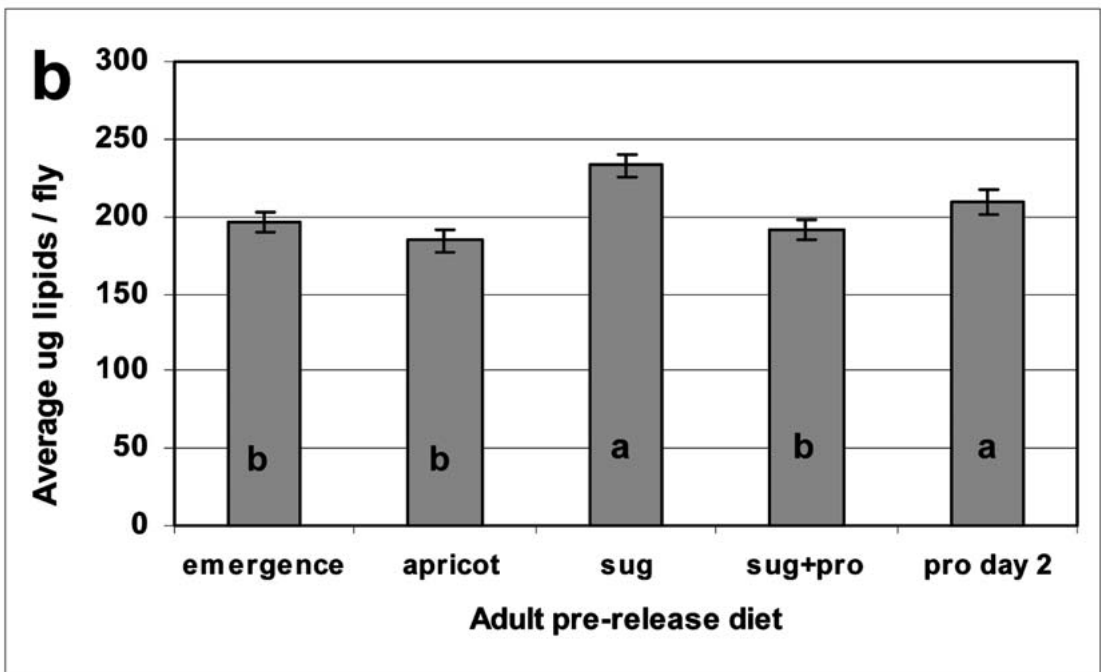
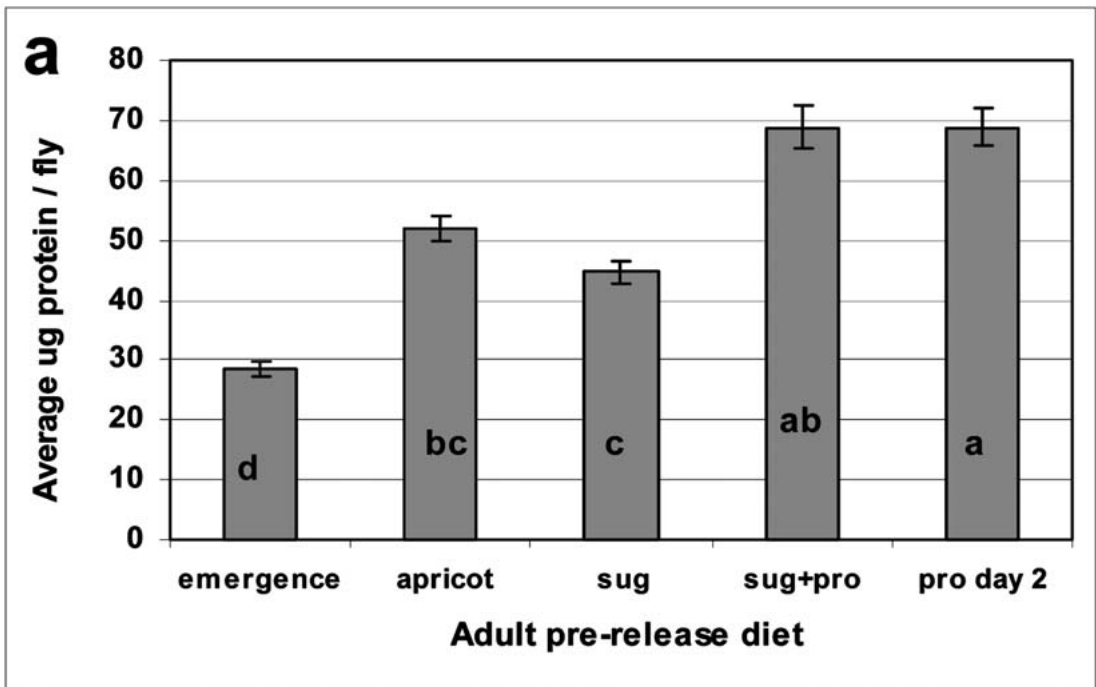


Fig. 2. Effect of pre-release diet on nutritional status of sterile male Mediterranean fruit flies. a. Protein. b. Lipid. Diets as in Fig. 1. Emergence refers to flies sampled within hours of leaving the puparium; all other flies were assayed at 4 d of age before release. Bars represent 2 standard errors. Different letters in columns denote a significant difference ( $P < 0.05$ ) in nutrient content.

that copula durations of sterile males (of the Maui-Med bisexual strain), mating with wild females in the laboratory, varied independently of male diet.

In the field enclosure experiment in Guatemala (see above), Shelly et al. (2003) found that copula duration varied with diet but not with exposure to ginger root oil. Significantly, protein-deprived (sugar-fed) males at the high elevation site copulated longer (average 141.4 min; range 11-255 min) than protein-fed males (120 min on average, range 15-225 min). Furthermore, copulations starting later in the day were significantly shorter than copulations starting early in the day (see also Vera et al. 2003). Assuming that males control copula duration, these findings suggest that males adaptively prolong copulation when the probability of their partner copulating again is highest and their own chances of being accepted as a mate, lowest.

**Female Remating.** A number of studies have shown that females first mated to a sterile male are more likely to remate than are females first mated to a wild male (e.g., Mossinson & Yuval 2003; Vera et al. 2003). Furthermore, Blay & Yuval (1997) found that females whose first mate was protein-deprived (sugar-fed) were more likely to remate than females first mated to a protein-fed wild male. However, in the only laboratory study combining the effects of diet and irradiation, Shelly & Kennelly (2002) found that the frequency of female remating varied independently of male diet regardless of whether her first mate was a wild or mass reared male. One difference between this study and those of Blay & Yuval (1997) and Mossinson & Yuval (2003) is that the first remating opportunity in the latter 2 experiments was offered on the day following mating, as opposed to 2 d following the initial mating in this study. It has been shown that male accessory gland secretions affect female receptivity (Jang 2002) and that their effect intensifies several days after mating (Miyatake et al. 1999; Mossinson & Yuval 2003). Although the effect of irradiation and post-teneral diet on these secretions has not been studied, the results of Shelly & Kennelly (2002) are consistent with a mechanism in which accessory gland secretions affect remating 2 d after the initial copulation.

The amount of sperm stored by female medflies determines, to a large extent, their short-term receptivity to further copulations (Miyatake et al. 1999; Mossinson & Yuval 2003). In comparison to the average ejaculate of wild males, which contains approximately 3,000 sperm (Yuval et al. 1996; Taylor et al. 2000), the ejaculate of sterile males contains fewer sperm, and this alone may prejudice the effectiveness of SIT. Furthermore, the amount of sperm stored by females significantly declines as the sterile male ages. Thus, sterile males are effective inseminators only on the 2-3 d following release. Wild females may copulate with older sterile males (but see Liedo et al.

2002), but they will probably rapidly copulate again, either on the same day or on the following one (Mossinson & Yuval 2003; Vera et al. 2003). Accordingly, the first few days following release are the critical period for sterile male effectiveness. If protein fed males can survive during this period, and bring competitive advantages to bear, the SIT operation will benefit.

#### SURVIVAL AND LONGEVITY

In the laboratory, no difference was documented in survival of sugar-fed or sugar and protein-fed sterile males when they had access to a natural source of food (apple slice) from the fifth d of life onwards. However, when these males were starved, a dramatic effect was observed. Diet significantly affected the probability of surviving starvation, with males who had previously also fed on protein dying significantly faster than sugar-fed males (Kaspi & Yuval 2000).

Maor et al. (2004) replicated these findings in a field enclosure experiment. A sample of males from each diet treatment was held in a cage within the field enclosure, either with or without food. After 2 d without food, most (>95%) of the flies (protein-fed and protein-deprived males) died. Conversely, most of the flies that had continuous access to a dry fig survived for as long as 18 d. Furthermore, post-teneral diet had a significant effect on the survival of the flies. Protein-deprived (sugar-fed) males survived longer without food, but protein-fed males survived longer when food was provided (Maor et al., 2004). Apparently, the ingestion of protein, while increasing male sexual activity, also affects male metabolism in such a manner that interruption of protein feeding has greater negative consequences than does interruption of a diet lacking protein. The protein rich diet provided to males may commit them metabolically to reproduction (Carey et al. 2002) by diverting resources to pheromone and accessory glands and energy to sexual advertisement. This commitment carries higher sexual rewards in some environments, but also the penalty of inability to weather periods of nutritional stress.

Recognizing the importance of olfactory cues containing  $\alpha$ -copaene in enhancing sexual performance of male medflies (Shelly 2001), the synergistic effect of olfactory cues and dietary regimes on survival was recently investigated (Levy et al. 2005). Working with wild flies from Israel and the VIENNA 7/Tol-2000 and (in Hawaii) VIENNA 4/Tol-94 strains, a series of experiments were conducted to determine how various diets, combined with exposure to volatiles containing  $\alpha$ -copaene, affect the ability of male medflies to withstand starvation.

Flies were presented with diets and olfactory stimuli from the moment of emergence for 4 d, simulating the pre-release period. From the

fourth d onwards, simulating release, diets and other stimuli were removed, and flies monitored until all had died. A comparison of the pooled survival rates of the 3 strains revealed that sterile males have a significantly lower ability to resist starvation compared to wild males. Furthermore, males from the 2 mass reared strains also differed significantly in their ability to endure starvation. Males from the strain shipped during approximately 72 h from Guatemala to Israel (VIENNA 7/Tol-2000) died significantly faster than males from the strain (VIENNA 4/Tol-94) tested in Hawaii without long-distance shipping.

The sugar diet, alone or in combination with  $\alpha$ -copaene, was associated with the highest ability to resist starvation, followed closely by the sugar + protein diet (again, alone or in combination with  $\alpha$ -copaene). Paradoxically, the apricot diet, which in some trials was associated with the best sexual performance (see above), contributed most significantly to the rapid death of the flies ingesting it. Furthermore, when the apricot diet was coupled with  $\alpha$ -copaene, rates of demise were faster yet (Levy et al. 2005).

#### PRE-RELEASE DIET AND FORAGING SUCCESS IN THE FIELD

The findings covered above prompt a key question—does pre-release diet affect the ability of sterile males to find food in the field? Results of a number of studies in field cages and enclosures shed some light on this matter.

Shelly & McInnis (2003) released protein + sugar-fed and protein-deprived (sugar-fed) sterile males from 2 different mass rearing strains into field cages containing a single guava tree. In 1 experiment, no food was provided, and all surviving males were collected after 2 d. Sixty one percent of males of the VIENNA 7/Tol-2000 strain from both diet treatments survived. In the other strain tested, Maui-Med bisexual strain, survival was lower overall, but similarly did not differ between diet treatments. In another experiment, food (papaya and orange slices) and water were placed in the field cages and surviving males collected 4 d later. Again, pre-release diet did not affect survival. Close to 40% of the males from both strains survived, irrespective of diet. The survival of males in the cages without food was probably due to the presence of bacteria or residual honeydew. The low survival after 4 d with food may be due to predation by ants, spiders or other invertebrate predators within the field cage.

To seek conclusive evidence for the ability of protein-fed sterile males to forage for food, Maor et al. (2004) determined the nutritional status of males sampled in leks within a field enclosure for 6 d following release. Post-teneral diet had no significant effect on the level of sugar in the flies, as most had high levels of carbohydrates that must

have been recently acquired. Diet did have a significant effect on the level of protein in the flies. Protein-fed males contained significantly more protein than protein-deprived (sugar-fed) males on the day of release and on the day following release. Finally, post-teneral diet had a significant effect on the level of lipids in the flies. Protein-fed males contained significantly less lipids than protein-deprived (sugar-fed) males on the day of release and on the first day following release. The level of lipids decreased significantly from d 0 to d 6 in both protein-fed and protein-deprived males.

These results demonstrate conclusively that when food is available at the release site, most sterile males are able to find and ingest it, irrespective of their pre-release diet.

#### PROSPECTS FOR THE FUTURE—MANIPULATION OF THE POST-TENERAL ENVIRONMENT

While some might hurry to conclude that sugar alone is the best diet for sterile males, we interpret the emerging picture in a different light. First, post-teneral diet does not affect foraging ability of sterile males (Shelly & McInnis 2003; Maor et al. 2004; Barry et al. 2003). Thus, if nutrition is available in the field, most sterile males will find it. Furthermore, due to their reduced ability to inseminate as they age, the sexual effectiveness of sterile males is diminished following the first 24-48 h after release (Taylor et al. 2001; Liedo et al. 2002). Such males may serve as distractions for wild females but will fail to inhibit their receptivity (Mossinson & Yuval 2003). Thus, it may be better to release relatively short-lived flies that are highly competitive, rather than long lived, sexually ineffective ones. Better still would be long-lived sterile males with prolonged competitive ability (e.g., McInnis et al. 2002). The enhancing effect of protein feeding, while found in all studies of wild flies, is not evident in all studies of sterile flies. This may be due to a strain-environment interaction, the ability of sugar fed flies to acquire protein by feeding on feces, pupal frass or the carcasses of dead conspecifics, or variations in the bacterial communities present in the guts of these insects.

Accordingly, more research is needed on the critical post-teneral period, a time when the sterile males and their environment are completely under the control of the SIT program personnel. An optimal protocol would incorporate diet, olfactory cues, and possibly hormonal and social stimuli as well. Its validation must be based on sexual performance of the sterile males in a competitive setting that emulates field conditions.

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