INFLUENCE OF DIET AND METHYL EUGENOL ON THE MATING SUCCESS OF MALES OF THE ORIENTAL FRUIT FLY, *BACTROCERA DORSALIS* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The chief objective of this study was to determine whether the inclusion of protein in the adult diet influences male mating success in the oriental fruit fly, *Bactrocera dorsalis* (Hendel). Previous studies on this species have shown that ingestion of methyl eugenol (ME) greatly enhances male mating performance. Accordingly, we also examined the interaction between adult diet and ME and investigated whether this chemical boosts the male mating success independent of diet. In trials conducted in field tents, we compared the mating frequency of control, protein-fed males (no ME) versus males (1) deprived of protein during the entire adult life, the pre-maturation period, or the post-maturation period and (2) not provided ME or provided ME 1 day before testing. Males deprived of protein completely or when immature (1-12 days old) obtained very few matings (<5% total matings) with or without ME feeding. Males provided protein as immature adults but deprived of protein as mature adults (>12 days old; no ME) also were competitively inferior to control males but achieved a significantly higher proportion (37%) of total matings than males in the preceding treatments. ME exposure boosted the mating success of these males slightly (40% of total matings) such that their mating frequency was not significantly different from control males. Additional tests showed that for treated males fed protein their entire adult life short-term (30 h) food deprivation resulted in a significant decrease in mating success, and feeding on ME did not boost the mating frequency of the food-deprived males. The implications of our findings for controlling *B. dorsalis* via sterile male releases are discussed.

Key Words: Diptera, Tephritidae, oriental fruit fly, diet, methyl eugenol, mating behavior

RESUMEN

El objetivo principal de este estudio fue el determinar si la inclusión de proteina en la dieta del adulto tiene una influencia en el éxito de apareamiento de los macho en la mosca oriental de fruta *Bactrocera dorsalis* (Hendel). Estudios anteriores sobre esta especie han mostrado que la ingestión de eugenol metil (EM) mejora sustancialmente la capacidad del apareamiento del macho. De consiguiente, nosotros tambien examinamos la interación entre la dieta del adulto y EM e investigamos si esta química aumentará el exito de apareamiento del macho independiente de la dieta. En pruebas llevadas a cabo en tiendas de campaña en el campo, nosotros comparamos la frecuencia de apareamiento del grupo control, machos alimentados con próteina (no EM) versus machos 1) privados de próteina durante su vida de adulto entera, el periodo de pre-maduración, o el periodo del pos-maduración y 2) no proveidos con EM o no proveidos con EM un dia antes de la prueba. Los machos completamente privados de proteina o que eran inmaduros (1-12 dias de edad) consiguieron muy pocos apareamientos (<5% de los apareamientos total) con o sin alimentar de EM. Los machos proveidos con proteina en el estadio inmaduro pero privados de proteina como adultos maduros (>12 dias de edad, sin EM) fueron inferiores en competir con los macho del grupo control pero lograron obtener una proporción significativamente mas alta (37%) de apareamientos total que los machos en los tratamientos anteriores. La exposición a EM aumentó el éxito del apareamiento de estos machos ligeramente (40% de todos los apareamientos) de tal manera que la frecuencia de apareamiento no fue significativamente diferente de la frecuencia de los machos en el grupo de control. Pruebas adicionales mostraron que, para los machos tratados con alimentos con próteina para toda su vida del adulto, la privación de alimentos a un corto plazo (30 h) resultaron en una diminución significativa en el éxito de apareamiento, y la alimentación de EM no aumentó la frecuencia de apareamiento de los machos privados de alimentos. Se discuten las implicaciones de nuestros hallazgos para controlar *B. dorsalis* por la via de liberación de machos estériles.

The quality of the adult diet, particularly the inclusion of protein, may influence the ability of male insects to attract females and obtain copulations as shown for certain species of Orthoptera (Andrade and Mason 2000; Scheuber et al. 2003) and Diptera (Stoffolano et al. 1995; Droney 1998). Recent data from two economically important genera of tephritid fruit flies (Diptera: Tephritidae) likewise reveal an important effect of dietary protein on male signaling and mating success (for

protein effects on female tephritids, see Cangussu & Zucoloto 1995; Jacome et al. 1999). Working with a wild population of Mediterranean fruit flies (medflies), *Ceratitis capitata* (Wiedemann), in Israel, Yuval et al. (1998) found that sexually active males (i.e., those participating in leks) contained more sugar and protein than sexually inactive (resting) males. In a follow-up study, Kaspi et al. (2000) observed wild medflies on field-caged host trees and found that protein-fed males spent more time emitting pheromone (see Papadopoulos et al. 1998 for a similar finding) and achieved more matings than protein-deprived males. In a study of wild medflies in Hawaii, Shelly et al. (2002) observed no effect of dietary protein on the frequency of pheromone calling but found that protein-fed males attracted more females than protein-deprived males and had a significant advantage in mating competition over protein-deprived males (see also Shelly & Kennelly 2002). In addition to this focus on dietary protein, several studies (Papadopoulos et al. 1998; Shelly & Kennelly 2003) have demonstrated the adverse effect of short-term food deprivation (18-24 h) on signaling activity and mating success of wild medfly males.

The effect of diet on the mating behavior of wild males also has been examined in several *Anastrepha* species. Aluja et al. (2001) reared wild males of 4 *Anastrepha* species on sugar, a mixture of sugar and protein, open fruit, or a mixture of sugar and bird feces and then compared males in the different diet treatments with respect to signaling activity and mating frequency. In 3 of the species, males fed the combination of sugar and protein displayed the highest frequency of pheromone-calling, and in two of these, males fed only sugar called significantly less frequently than conspecifics fed other diets. In the same 3 species, males fed the sugar and protein diet obtained significantly more matings than males fed other diets. In only 1 species, *A. ludens* (Loew), did diet have no effect on male sexual activity.

The chief objective of the present study was to determine whether dietary protein has a similarly strong effect on male mating success in the Oriental fruit fly, *Bactrocera dorsalis* (Hendel). Previous studies on *B. dorsalis* (e.g., Shelly & Dewire 1994) have demonstrated that ingestion of methyl eugenol (ME), a powerful male attractant occurring naturally in many plant species (Tan & Nishida 1996), dramatically increases male mating success, apparently owing to the incorporation of ME metabolites in the sex pheromone (Nishida et al. 1987) and the subsequently heightened attractiveness of the olfactory signal (Shelly & Dewire 1994). The effect of ME feeding on male reproductive performance has been investigated only within the context of protein-rich, adult diets. Consequently, we also examined the interaction between diet and ME feeding to deter-

mine whether ME feeding similarly boosts the mating performance of protein-deprived males. Finally, in light of the aforementioned results for the medfly, we measured the effect of food deprivation on the mating success of *B. dorsalis* males. Implications of our findings for controlling *B. dorsalis* via sterile male release are discussed. For background information on the mating behavior of *B. dorsalis*, see Fletcher (1987) and Shelly and Kaneshiro (1991).

Materials and Methods

Study Flies

All flies used in the present study were from a laboratory colony started with 600-800 adults reared from papayas (*Carica papaya* L.) collected near Hilo, HI. The colony was maintained in a screen cage (l:w:d, $1.2 \times 0.6 \times 0.6$ m) and provided a mixture (3:1, wt:wt) of sugar (sucrose) and hydrolyzed protein (enzymatic yeast hydrolysate) and water *ad libitum* and papayas for oviposition. Infested papayas were held over vermiculite, and the pupae were sifted from vermiculite 16-18 days later. Adults used in mating trials were separated by sex within 48 h of eclosion, well before reaching sexual maturity at ≈ 15 days of age (TES, unpublished data), and held in screen-covered, plastic buckets (volume 5 liters with a cloth sleeve to allow transfer of flies, food, and water; 100-125 individuals per bucket) with ample food and water. Flies were held at 24-28°C and 60-90% RH and received natural and artificial light under a 12:12 (L:D) photoperiod. When used in the experiments, the flies were 3-7 generations removed from the wild.

Competitive Mating Tests

Four mating experiments were conducted in which males subject to different rearing regimes competed for copulations. All tests used mature individuals of both sexes (males: 21-25 days old; females: 21 - 29 days old). In each experiment, the control males were fed the sugar-protein mixture (hereafter termed the SP diet) and water continuously during their entire adult life (as were all females tested) and never given access to ME. The food mixture was placed in a small Petri dish (5 cm diameter), and water was provided via a cotton wick emerging from a covered, plastic cup. Both the food and water were changed every 5-7 days.

Treated males were subject to the following conditions.

Experiment 1a: Treated males were fed sugar-agar exclusively throughout their entire adult life (i.e., these males were protein-deprived) and were not given access to ME. No water cup was provided. The sugar-agar diet (hereafter termed the

S diet) was prepared following the recipe of the California Preventative Release Program and included water (84.66% by weight), sugar (14.57%), agar (0.76%), and methyl parabin (0.01%). A block of the sugar-agar (l:w:h, approximately $6 \times 3 \times 2$ cm) was placed directly on the screen-cover of the bucket and was replaced every 2-3 days.

Experiment 1b. Same as above, except that treated males were given access to ME (supplied by FarmaTech Intl. Corp., Fresno, CA). In this, and all subsequent experiments involving ME exposure, treated males were given access to ME on the day before testing. Using a micropipette, we applied 100 μl of ME to a cotton wick (held vertically by insertion through a hole in the lid of a plastic cup), which was then placed in a bucket holding 80-100 males. The chemical was introduced between 1000-1300 hours and removed 1 h after placement. Feeding activity was not monitored in this study, but in a previous study (Shelly 1997) over 90% of mature males were found to feed on ME within a 1-h interval.

Experiment 2a. Treated males were fed the S diet from 1-12 days of age and were then provided the SP diet until tested (hereafter termed the S-SP diet). As males do not attain sexual maturity until about 15 days of age (TES, unpublished data), this treatment provided sugar only to immature males but sugar and protein to sexually mature males. Treated males were not given access to ME.

Experiment 2b. Same as experiment 2a, except that treated males were given access to ME following the above protocol.

Experiment 3a. Treated males were fed the SP diet and water from 1-12 days of age and were then provided the S diet until tested (hereafter termed the SP-S diet). Here, we reversed the treatment of experiment 2a and provided sugar and protein to immature males but sugar only to mature males. Treated males were not given access to ME.

Experiment 3b. Same as experiment 3a, except that treated males were given access to ME following the above protocol.

Experiment 4a. Treated males were fed the SP diet during their entire life but were starved for approximately 30 h prior to testing. Treated males were not given access to ME. As noted above, *B. dorsalis* is sexually active at dusk, and consequently we removed the food (but not the water cup) at noon on the day before testing.

Experiment 4b. Same as experiment 4a, except that treated males were fed ME. In this case, ME was introduced at 1100 hours, and starvation was initiated upon termination of the exposure period.

Competitive mating trials were conducted between August 2004-January 2005, in field cages (3 m diameter; 2.5 m height) at the USDA-ARS laboratory, Honolulu (air temperature: 25-30°C; RH: > 60%). The tents each contained two artificial trees whose leaves resembled those of *Ficus benjamina* L. Each tree was approximately 2 m tall and bore 700-800 leaves. Males perform the normal suite of reproductive behaviors on these trees, and matings occur as frequently as on potted host trees (TES, unpublished data). Groups of 75 control males, 75 treated males, and 75 females were released approximately 2 h before sunset (between 1600-1700 hours depending on the test date). For a given trial, we marked both control and treated males 1 day prior to testing by cooling them for several minutes and placing a dot of enamel paint on the thorax. The cages were monitored from 1 h before sunset until approximately 30 min after sunset with a flashlight. Mating pairs were collected in vials and returned to the laboratory where the males were identified. Eight replicates were conducted for experiments 1-3, and 10 replicates were conducted for experiment 4.

Non-Competitive Mating Tests

Because adult diet had a profound effect on male mating success (see below), we conducted a series of non-competitive mating tests to assess the mating propensity of males exposed to different dietary regimes. To expedite data collection, these tests were run in our laboratory by placing 10 females (maintained on the SP diet) and 10 males of a given treatment in plexiglass cages (l:w:h, $40 \times 30 \times 30$ cm) approximately 2 h before sunset and scoring the number of matings 2-3 h after sunset. Cages were placed near a west-facing window and exposed to natural light (room lights were extinguished when flies were placed in the cages). When tested, males were 21-24 days old, and females were 23-29 days old. For a given male treatment, 6-10 cages were run per day over 3-5 different days for a total of 30 replicates (cages) per treatment.

Non-competitive mating tests were conducted with males subject to treatments identical to those described above for the competitive mating trials and included control males and treated males as described for experiments 1-3. Test males were subject to the following conditions.

Experiment 5. Males were fed the SP diet during their entire life and were not given access to ME.

Experiment 6a. Males were fed the S diet during their entire life and were not given ME.

Experiment 6b. Same as above, but males were given access to ME.

Experiment 7a. Males were fed the S-SP diet and were not given access to ME.

Experiment 7b. Same as above, but males were given access to ME.

Experiment 8a. Males were fed the SP-S diet and were not given access to ME.

Experiment 8b. Same as above, but males were given access to ME.

Diet and Male Survival

Survival was compared among males maintained on the SP, S, S-SP, or SP-S diets. For each diet, 20 males (1 day old) were placed in screen cages (30 cm cubes) with the appropriate diet under the laboratory conditions described above. All diets were presented in small Petri dishes; water was provided with all diets except the S diet. Food and water were changed every 2-3 days. Cages were checked midday every day for 40 days, and dead males were removed during the daily observations. Ten cages were observed for each diet type.

Statistical Analyses

For the competitive mating experiments, we first compared the number of matings obtained per replicate between control and treated males using a t-test as the assumptions of normality and equal variances were met in nearly all cases (the exceptions were experiments 1a, 1b, and 2a, respectively, and here data were normalized via $\log_{10}$ [x + 1] transformation). Because there was significant variation in the total number of matings observed per replicate among the different experiments ($F_{7, 60} = 5.2$, P < 0.001, ANOVA; presumably owing to slight variation in weather conditions), we compared mating performance among the different treatments using the proportion (arc sine transformed) of the total matings obtained per replicate in an ANOVA. For the noncompetitive mating experiments, mating numbers were compared among male treatments by the Kruskal-Wallis test (a logarithmic transformation failed to normalize the data). For male survivorship data, the number of survivors was plotted against time for the different treatments, and slopes of the regression lines were compared by ANCOVA following Zar (1996). As described below, multi-group comparisons involving mating frequency or survivorship revealed significant variation in all cases, and consequently the Tukey test was run to identify significant differences in specific pair wise comparisons. For survivorship, the test statistic q was calculated according to Zar (1996).

RESULTS

Competitive Mating Tests

Results of the competitive mating trials are presented in Table 1. The most striking finding was the dramatic, negative effect of protein-depri-

TABLE 1. EFFECTS OF ADULT DIET, METHYL EUGENOL (ME), AND STARVATION ON THE MATING SUCCESS OF *B. dorsalis* MALES IN COMPETITIVE MATING TRIALS.

Experiment	Treatment Diet	ME	Starvation	Male type	Number of Matings[1]	t[2]	% Total Matings[3]
1 a	S	no	no	Control	20.4 (3.7)	12.3***	
				Treated	0.8 (1.1)		2.8[a]
1 b	S	yes	no	Control	21.0 (3.4)	16.6***	
				Treated	0.7 (0.7)		3.2[a]
2 a	S-SP	no	no	Control	17.1 (2.4)	13.2***	
				Treated	0.9 (1.0)		4.7[a]
2 b	S-SP	yes	no	Control	13.6 (3.4)	0.2[NS]	
				Treated	13.0 (6.2)		47.0[b]
3 a	SP-S	no	no	Control	18.9 (4.6)	3.3**	
				Treated	11.2 (4.5)		37.5[b]
3 b	SP-S	yes	no	Control	16.8 (4.8)	1.2[NS]	
				Treated	13.2 (5.2)		40.5[b]
4 a	SP	no	yes	Control	12.8 (4.5)	2.8*	
				Treated	7.3 (3.8)		35.6[b]
4 b	SP	yes	yes	Control	17.1 (8.9)	2.5*	
				Treated	8.7 (5.5)		35.9[b]

[1]Values represent average numbers (±1 sd) of matings per replicate and average proportion of total matings obtained by treated males; 8-10 replicates were conducted per experiment.
[2]Tests compare control and treated groups for a given experiment, where significance levels are: ***P < 0.001; **P < 0.01; *P < 0.05; [ns]not significant.
[3]Proportions followed by the same letter are not significantly different at P = 0.05, tukey test.

vation on male mating success. Males fed the S diet their entire life obtained, on average, less than 1 mating per replicate without (experiment 1a) or with (experiment 1b) prior access to ME. Males maintained on the S-SP diet and not provided ME (experiment 2a) had a similarly low mating success. Treated males in these experiments accounted for a similar proportion (3%-5%) of the total matings. In contrast to the S diet, males fed the S-SP diet and then exposed to ME (experiment 2b) displayed a significant increase in mating success and were, in fact, competitively equivalent to control males.

Males maintained on the opposite dietary regime, SP-S, and denied access to ME (experiment 3a) were competitively inferior to control males, but they obtained, on average, a significantly higher proportion of matings per replicate than S-SP males denied ME (experiment 2a; 38% vs. 5%, respectively). Exposure to ME increased the mating success of males reared on the SP-S diet (experiment 3b), and the mean number of matings obtained by these males was similar to that observed for the control males. Relative to the S-SP diet used in experiment 2, however, the effect of ME exposure with the SP-S diet was slight: males fed the SP-S diet and given ME or denied ME accounted for a similar proportion of the total matings (40% versus 37%, respectively).

Starvation had a strong negative effect on mating frequency. Control males had a mating advantage over starved males (previously maintained on the SP diet) independent of ME feeding (experiments 4a and 4b). Starved males denied or provided ME obtained the same proportion (36%) of the total matings.

Non-Competitive Mating Tests

Results from the non-competitive mating tests mirrored those described above for the competitive tests (Table 2). Significant variation in mating frequency was observed among male treatments (H = 64.8, *df* = 6, *P* < 0.001). Males on the S and S-SP diets exhibited mating frequencies that were significantly lower than those observed for the SP or SP-S diets. Over the 60 replicates involving the S diet (i.e., experiments 6a and 6b combined), we observed a total of only 3 matings. Similarly, only 5 matings were recorded for males fed the S-SP diet independent of ME exposure. Males on the SP diet displayed the highest mating frequency, although this was not significantly different from males on the SP-S diet after ME exposure.

Male Survivorship

Significant variation in slope existed among the different diet treatments ($F_{3, 28}$ = 5.9, *P* = 0.007), with survival rate being greatest for the

TABLE 2. EFFECTS OF ADULT DIET AND METHYL EUGENOL (ME) ON THE MATING FREQUENCY OF *B. DORSALIS* MALES IN NON-COMPETITIVE MATING TRIALS.

| Experiment | Treatment | | Number of Matings | |
	Diet	ME	Mean	Median[1]
5	SP	no	3.8 (1.5)	4[a]
6 a	S	no	0.1 (0.2)	0[c]
6 b	S	yes	0.1 (0.2)	0[c]
7 a	S-SP	no	0.2 (0.5)	0[c]
7 b	S-SP	yes	0.2 (0.4)	0[c]
8 a	SP-S	no	2.6 (1.3)	3[b]
8b	SP-S	yes	2.9 (1.4)	3[ab]

[1]Mean (±1 sd) and median numbers of matings are given over 30 replicates per experiment. Medians followed by a common letter are not significantly different at *P* = 0.05, Tukey test.

SP diet, lowest for the S diet, and intermediate for the SP-S and S-SP diets (Fig. 1). Multiple comparison tests revealed that survival rate for the SP diet was significantly greater than for the S or SP-S diets but not significantly different from the S-SP diet. No significant differences were detected in pair wise comparisons among the S, SP-S, and S-SP diets. The simple linear regression equations were: SP: y = 18.5-0.16×, r² = 0.89; S: y = 18.6-0.31×, r² = 0.97; S-SP: y = 18.1-0.22×, r² = 0.90; SP-S: y = 18.8- 0.26×, r² = 0.94.

DISCUSSION

The present study demonstrates a strong effect of diet quality on the mating success of *B. dorsalis* males. The inclusion of hydrolyzed protein in the
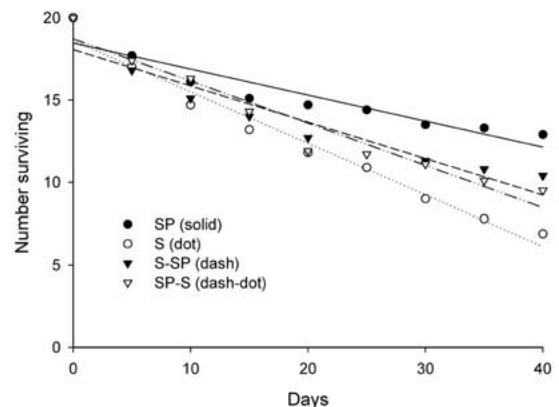

Fig. 1. Survivorship of males maintained on different diet regimes. Points represent means of 10 cages per diet treatment.

adult diet was very important for mating; males fed the sugar-agar gel exclusively achieved very few matings in competitive or even non-competitive conditions. The low number of copulations observed in the non-competitive situation further suggests that males were not sexually active at all or were producing signals unattractive to females. Interestingly, males reared on the S diet until day 12 and then switched to the SP diet for 8-12 days before testing (and not given ME) performed as poorly as males reared on the S diet exclusively, and this was evident in both competitive and noncompetitive situations. Males subject to the opposite treatment (SP-S) had a lower mating success than control males in the competitive trials but nonetheless accounted for a much higher proportion of the total matings than did the S or S-SP males. Results from the starvation treatment further revealed that males deprived of the PS diet for a single day had reduced mating success relative to males continuously fed the same diet.

Dietary protein appears to have a greater effect on mating success for wild males of *B. dorsalis* than for wild males of *C. capitata*. Whereas sugar-fed males of *B. dorsalis* rarely mated, sugar-fed males of *C. capitata* accounted for approximately 33% (Kaspi et al. 2000) and 40% (Shelly et al. 2002) of the total matings in competition with protein-fed males. The impact of dietary protein varied greatly among *Anastrepha* species (Aluja et al. 2001). As in *B. dorsalis*, males of *A. serpentina* (Wiedemann) and *A. striata* Schiner that were fed a sucrose solution only mated very infrequently (<10% total matings). In contrast, based on non-competitive mating trials, males of *A. obliqua* (Macquart) fed sugar only still mated at nearly half the rate as protein-fed males, and no diet effect at all was detected for *A. ludens*.

The present findings provide only a broad description of the impact of adult nutrition on male mating success, and additional tests are required to determine more specifically the effect of intermittent protein-feeding on male performance. In particular, future studies should address the question of whether, among sexually mature males, mating success declines with time since the last protein meal. In other words, while the present study showed that both (i) a sugar-only, post-maturation diet and (ii) starvation from a protein-rich diet reduced male mating success, it did not investigate the impact of more realistic feeding regimes, where, for example, males may locate sugar-rich foods more or less continuously but protein-rich foods only irregularly.

In addition to demonstrating the importance of dietary protein, our study also provided information regarding the interaction between diet and ME on male mating success. Among continuously fed males, ME had no effect on mating performance for males that were fed the S diet exclusively and only a slight effect on males fed the SP-S diet. However, ME dramatically boosted mating success under the S-SP diet: ME-fed males obtained an average of 47% of all matings per replicate (experiment 2b) compared to only 5% for ME-deprived males (experiment 2a). Although ME exposure under the S-SP diet yielded equivalent mating success between treated and control males, prior studies (Shelly & Dewire 1994; Shelly & Nishida 2004) have shown that, among males fed the SP diet exclusively, individuals provided with ME obtain approximately 2/3 of all matings in competition against ME-deprived individuals. Thus, ME exposure under the S-SP diet apparently compensated for a low quality diet, but it did not confer a mating advantage as evident under the SP dietary regime. Interestingly, ME feeding did not offset the adverse effect of starvation (from the SP diet; experiment 4b) on male mating success. It thus appears that the ability to locate protein-rich food is essential for *B. dorsalis* males, because temporary deprivation of the SP diet not only reduced mating performance but also eliminated the potential benefit associated with ME ingestion.

In conclusion, our study is potentially relevant to control programs, such as that ongoing in Thailand, that utilize the sterile insect technique (SIT) to suppress or eradicate infestations of *B. dorsalis*. Our tests were performed exclusively on flies from a relatively "young" laboratory colony, and additional work is required to determine whether the composition of the adult diet similarly influences the mating competitiveness of males from long-established, mass-reared strains of *B. dorsalis*. In the Mediterranean fruit fly, for example, inclusion of protein in the adult diet invariably results in increased mating success of wild males (Yuval et al. 1998; Kaspi et al. 2000; Shelly et al. 2002), whereas the results for mass-reared males have been inconsistent (compare Blay & Yuval 1997; Kaspi & Yuval 2000 with Shelly and Kennelly 2002; Shelly and McInnis 2003). Clearly, however, if adult diet similarly affects mass-reared *B. dorsalis* males, an effective use of the SIT would require inclusion of protein in the pre-release diet.

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