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## EFFECT OF NITROGEN-CONTAINING DIETARY SUPPLEMENTS ON THE MATING SUCCESS OF STERILE MALES OF THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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### ABSTRACT

In Sterile Insect Technique (SIT) programs, mass-reared males of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), are maintained on a sugar-agar diet before their release into the environment. Several studies suggest that nitrogenous dietary supplements improve the mating competitiveness of the sterile males, thus increasing the cost effectiveness of SIT. Other research, however, has not supported this notion. Here, we further investigate the potential usefulness of nitrogen-containing diet additives by examining the effect of 4 different nitrogenous materials—yeast hydrolysate, urea, whey protein, and honey—on the mating success of sterile *C. capitata* males. These materials were mixed directly with the sugar-agar diet in varying concentrations (1, 5, or 10%). Trials conducted in outdoor field-cages generated 2 consistent results across all diets: (1) neither the type nor concentration of nitrogenous material used elevated the mating success of sterile males above that observed for the standard sugar-agar diet, and (2) wild males invariably had a significant mating advantage over sterile males, accounting for an average of 81% of the total matings per replicate. In addition, a field comparison of short-term dispersal from a central release point revealed no significant difference between sterile males fed (i) the sugar-agar diet or (ii) the sugar-agar diet supplemented with yeast hydrolysate (10%) in either the number or spatial distribution of recaptured individuals. Our results do not support the proposal that pre-release nitrogenous dietary supplements improve the field performance of sterile *C. capitata* males in SIT programs.

**Key Words:** *Ceratitis capitata*, Sterile Insect Technique, diet, sexual competitiveness, dispersal

### RESUMEN

En programas con técnicas de insecto estéril (TIE), los machos criados masivamente de la mosca mediterránea (*Ceratitis capitata* Wiedemann) de la fruta, fueron mantenidos con una dieta de azúcar-agar antes de ser liberados al medio ambiente. Varios estudios sugieren que los suplementos con nitrógeno en la dieta mejora la habilidad de los machos estériles para aparearse, a su vez aumentando la eficiencia de los costos del programa de TIE. Sin embargo, otro estudio no ha suportado este resultado. Aquí, investigamos el uso potencial de los aditivos de dieta que contienen nitrógeno con el evaluo del efecto de 4 materiales de nitrógeno diferentes—levadura hidrolizada, urea, proteína de suero y miel—sobre el éxito de machos estériles de *C. capitata* para aparearse. Se mezclaron estos materiales directamente con la dieta de azúcar-agar en varias concentraciones (1, 5 o 10%). Las pruebas que se realizaron en jaulas del campo mostraron 2 resultados en todas las dietas: (1) ni la clase o la concentración de material de nitrógeno usada elevaron el éxito de los machos estériles para aparearse sobre el observado con la dieta de azúcar-agar y (2) los machos salvajes invariablemente expresaron una ventaja significativa mayor en el apareamiento que los machos estériles, representando un promedio de 81% del total de los apareamientos por repetición. Además, una comparación en el campo de la dispersión de corto plazo de los machos estériles liberados de un punto central reveló ninguna diferencia significativa entre los machos estériles alimentados de (i) la dieta de azúcar-agar o (ii) la dieta azúcar-agar suplementada con levadura hidrolizada (10%) en tanto el número o distribución espacial de los individuos capturados. Nuestros resultados no apoyan la proposición que los suplementos de nitrógeno en la dieta de los machos antes de ser liberados mejoran el desempeño en el campo de los machos estériles de *C. capitata* en programas de TIE.

In a seminal paper, Yuval et al. (1998) reported that the nutritional status of male Mediterranean fruit flies (medflies), *Ceratitis capitata* (Wiedemann), affected their ability to participate in mating aggregations or leks. Biochemical analysis of field-captured males showed that lekking males, which were actively signaling (i.e., emitting sex pheromone) from leaf territories when collected,

contained higher levels of protein and sugar than resting males, which were perching outside the lek and not signaling. This result garnered considerable attention because it hinted that improvements to the adult diet might effectively increase the mating competitiveness of mass-reared, sterile males used in the Sterile Insect Technique (SIT) against the medfly.

Recently, Yuval et al. (2007) reviewed the literature pertaining specifically to the effects of protein as an adult dietary supplement on the mating success of *C. capitata* males. Barry et al. (2007) reported that yeast hydrolysate, the purported protein source used in these studies, actually contains very little protein and is more accurately identified as a nitrogen or amino acid source. Work on wild flies has uniformly demonstrated that males given access to sugar (sucrose) + yeast hydrolysate gain an advantage in mating competition over males fed sugar only (Kaspi et al. 2000; Shelly & Kennelly 2002; Shelly et al. 2002). In contrast, studies on sterile males have generated inconsistent results. For example, Kaspi & Yuval (2000) found that sterile males fed a sugar-agar gel (the standard pre-release, adult diet used in SIT programs; e.g., Dantas et al. 2004; Barnes et al. 2004) supplemented with yeast hydrolysate (9%) achieved significantly more copulations than males fed the sugar-agar gel alone. In contrast, in studies conducted in Hawaii (Shelly & Kennelly 2002; Shelly & McInnis 2003; Shelly et al. 2006), sterile *C. capitata* males provided sugar only obtained similar numbers of matings as sterile males fed sugar and yeast hydrolysate. Mating trials performed in Guatemala further suggested that the effects of yeast hydrolysate may vary with environmental conditions: supplementing sugar with yeast hydrolysate significantly increased the mating performance of sterile males at a cool, high elevation site but had no detectable effect at a warmer, low elevation site (Shelly et al. 2004).

The present study further investigates the potential effectiveness of nitrogen-containing adult diet supplements in enhancing the mating success of sterile *C. capitata* males. Unlike previous studies (except Kaspi & Yuval 2000), we presented the nitrogen sources mixed in the standard sugar-agar gel and not separately from granular sugar. Additionally, whereas previous studies used yeast hydrolysate as a nitrogen source exclusively (excepting Maor 2004 et al., who used dried apricots), the experiments described herein were performed not only with yeast hydrolysate, but with several alternate nitrogen sources, namely urea, whey protein, and honey. Also, these supplements were added in different concentrations to examine possible dose-dependent effects. Finally, in addition to mating performance, we compared dispersal between sterile males fed the sugar-agar gel and sterile males fed the sugar-agar gel with yeast hydrolysate added.

## MATERIALS AND METHODS

### Study Insects

Wild flies were reared from infested coffee, *Coffea arabica* L., berries collected near Haleiwa, Oahu. Fruits were held over vermiculite and lar-

val development proceeded *in situ*. Pupae were sifted from the vermiculite 7-9 d after fruit collection, and adults used in this study were separated by sex within 2 d of eclosion, well before reaching sexual maturity at 6-8 d of age. Adults were held in plastic buckets covered with nylon screening (volume 5 L; 100-125 flies per bucket). Wild flies were provided with a mixture (3:1 v/v) of sugar and yeast hydrolysate and water *ad libitum*, held at 24-28°C and 60-90% RH, and received both natural and artificial light in a 12:12 (L:D) photoperiod. When used in the present study, the wild flies were 3-6 generations removed from the wild.

Mass-reared males were from a temperature sensitive lethal (*tsl*) genetic sexing system (Vienna-7/Tol-99) and were reared by the California Department of Food and Agriculture (CDFA) Hawaii Fruit Fly Rearing Facility, Waimanalo, Oahu. In rearing this strain, eggs are exposed to high temperature, which selectively kills female embryos and thus allows production and release of males only (Franz et al. 1994). Pupae were obtained 2 d before eclosion after dusting with pink fluorescent dye (particles of which adhere to the emerging adults, thus serving as a strain marker) and gamma irradiation at 150 Gy with a <sup>137</sup>Cs source. On a given day, pupae were placed in 4 screen-covered plastic buckets (approximately 150 pupae per bucket), and emerging adults were provided a prescribed diet (see below) and water and maintained under the same conditions described above.

### Dietary Treatments of Mass-Reared Males

As described below, we compared the mating success of mass-reared males that were fed the standard sugar-agar diet or a sugar-agar diet to which varying amounts of nitrogen-containing material were substituted for the corresponding amount of sugar. The sugar-agar gel was prepared following the recipe used by the CDFA's Medfly Preventative Release Program (95.02% sugar, 4.91% agar, and 0.07% methyl paraben; 1 L of water per 181 g of dry matter; I. Walters, pers. comm.). Four different nitrogenous substances were tested: yeast hydrolysate (USB Corporation, #216-765-5000, containing protein and amino acids, Tsiropoulos 1978; Morton & Bateman 1981; Barry et al. 2007), urea (Mallinckrodt Baker, Inc., #8642-12; NH<sub>2</sub>CONH<sub>2</sub>), whey protein (BIO-CHEM® Sports), and honey (containing amino acids plus vitamins and minerals, White et al. 1962; Hermosín et al. 2003). All 4 of these substances were added at 3 different levels, 1%, 5%, and 10% of the total amount of sugar (with sugar reduced by the equivalent amount), after the sugar-agar gel had cooled below 45°C to avoid protein denaturation.

Food was placed on the plastic buckets on the day of adult emergence (i.e., 2 d after pupal placement). Slabs of diet (10 × 6 × 3 cm, l:w:h) were

placed on the screen-covering, overlain with a moist paper towel to reduce desiccation, and replaced every other day. Among the 4 buckets prepared, 1 received the standard sugar-agar gel, and the remaining 3 buckets each received the same nitrogen-enhanced diet at 1, 5, or 10% concentration, respectively.

#### Mating Trials

Mating trials were conducted between Jul-Oct, 2006, at the USDA-ARS laboratory in Honolulu. Groups of 75 wild females (10-14 d old), 75 wild males (8-13 d old), and 75 mass-reared males (5-6 d old) were released between 0800-0830 h in field cages (2.5 m in height, 3.0 m in diameter) that contained a single artificial tree (2 m tall with ~500 leaves resembling those of *Ficus benjamina* L.; Silkwood Wholesale, Honolulu, HI). Artificial trees were used, because they provide a chemically neutral substrate on which the flies display the entire complement of natural activities. The cages were monitored for 4 h, mating pairs were collected in vials, and the males identified under a UV (black) light (*tsl* males—pink dye; wild males—no dye). All unmated individuals were removed from the cages after the trials; trees were not cleaned between trials. At least 20% of the females mated in all trials, consequently all data were included in the analysis per standard quality control guidelines (FAO/IAEA/USDA 2003).

On a given day, tests were run in 4 cages. One cage contained *tsl* males maintained on the standard sugar-agar diet, and each of the remaining 3 cages contained males maintained on a particular concentration (i.e., 1, 5, or 10%) of the same nitrogen source. Diet treatments were randomly assigned to specific tents on each test day. Weekly, we conducted mating trials on 2-4 d and rotated (on a random basis) the nitrogen source tested. Ten replicates were conducted for each nitrogen source-concentration combination. Over the study period, air temperature ranged between 26-32°C during the trials.

#### Dispersal

The potential effect of diet on male dispersal was examined through a mark-recapture study conducted between Jul-Oct 2007 in a coffee field near Haleiwa, Oahu. The mass-reared pupae from a daily shipment were divided into 2 groups, which were dusted with dyes of different colors. Following irradiation, the differently colored pupae were placed in separate storage boxes of the same type used in CDFA's Medfly Preventative Release Program (so-called PARC boxes, 60 × 48 × 33 cm, l:w:h). Within each box, 100 mL of pupae were placed in each of 6 paper bags for an approximate total of 36,000 pupae (~60 pupae per mL). On the day of peak adult emergence, we placed the sugar-

agar diet on the screen-covered top of one box and yeast hydrolysate-supplemented diet on the other (only the 10% concentration was tested). Food slabs (20 × 15 × 3 cm, l:w:h) were overlain with moist paper and replaced as previously described.

Four d after peak emergence, males from both boxes were released between 0900-1000 h at a designated point in the coffee field. At the same time 2 d later, we placed trimedlure-baited Jackson traps at the release point, at points 25 m from the release point along the 4 cardinal directions (N, S, E, and W), and at points 50 m from the release point along the 4 cardinal directions and lines offset 45° from the cardinal headings (i.e., NE, SE, SW, NW). Thus, 12 traps were arranged in 2 concentric circles (4 and 8 traps in the inner and outer circles, respectively) about a central trap (the release point). In baiting the traps, we applied 2 mL of trimedlure (a male attractant) to a cotton wick, which was suspended in a perforated plastic basket above a sticky insert placed inside the delta-shaped, trap body. Traps were suspended 1.5-2.0 m above ground in shaded sites within the canopy of coffee bushes and were left in place for 24 h. The identity of trapped males was scored under a UV light. Seven replicates were run with the same release point and trap sites. Successive releases were separated by 7 d, and the dye colors used to mark males were alternated weekly.

#### Statistical Analysis

Multiple comparisons regarding the numbers of matings obtained by wild and sterile males were made among (i) different treatments for a given nitrogen source or (ii) different nitrogen sources with 1-way ANOVA. Data from trap captures were analyzed by a 2-way ANOVA with male diet and distance from release point as the main factors. Because circumference trap 'density' was equal for the 25 (4 traps/157 m) and 50 m (8 traps/314 m) radius circles, data were combined over all traps for each distance. Data for both mating and trapping trials met parametric assumptions. Pair wise comparisons were made with a paired *t* test (proportional data were arc sine transformed) or the nonparametric Mann-Whitney test (test statistic *T*).

### RESULTS

#### Mating Trials

The results of the mating trials were remarkably consistent over all of the adult diets tested (Fig. 1). Over all treatments, the average of number of matings obtained per replicate varied only between 30.5-37.5 for wild males and 6.5-9.0 for mass-reared males. As this finding implies, the numbers of matings obtained by wild and *tsl* males, respectively, varied independently among

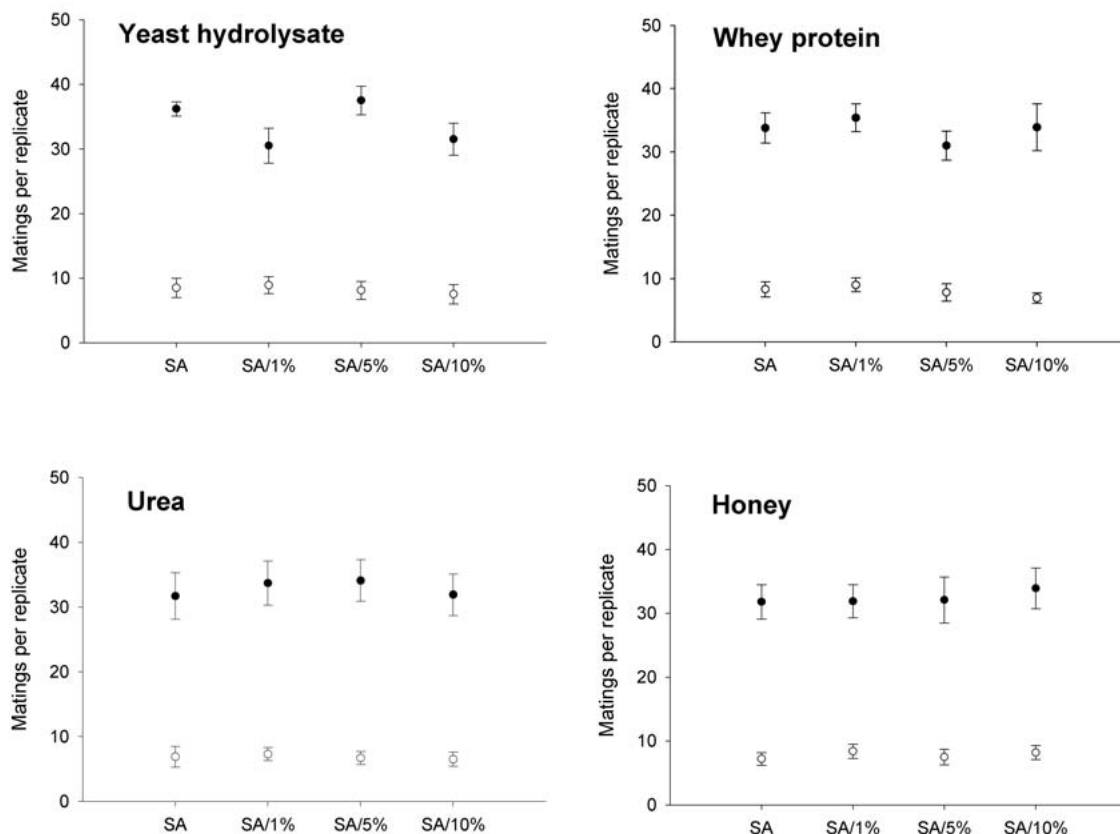


Fig. 1. Mating success of wild (●) and mass-reared, sterile, *tsl* (○) males of *Ceratitis capitata* in field cage trials. Points represent the average ( $\pm 1$  SE) number of matings per replicate ( $n = 10$ ). Wild males were always fed sugar and yeast hydrolysate. Sterile males were fed standard sugar-agar (SA) or the standard sugar-agar to which 1% (SA/1%), 5% (SA/5%), or 10% (SA/10%) of a given nitrogenous material was substituted for sugar.

(i) the 4 different treatments involving the same nitrogen source ( $F$  tests, where  $df = 3, 36$  and  $P > 0.05$  in all 8 cases based on 2 male types  $\times$  4 nitrogen sources) and (ii) the 4 different nitrogen sources (trials involving standard sugar-agar diet excluded; data combined across the 3 concentrations tested for each nitrogen source; wild males:  $F_{3,116} = 0.05$ ,  $P = 0.98$ ; sterile males:  $F_{3,116} = 1.05$ ,  $P = 0.37$ ). In other words, neither the type nor relative amount (from 0-10%) of nitrogenous material mixed with the standard sugar-agar diet appeared to influence the mating success of *tsl* males. Based on data from all trials, wild males obtained a significantly greater number of matings per replicate than *tsl* males (33.2 versus 7.7, respectively,  $T = 12929.5$ ,  $n_1 = n_2 = 160$ ,  $P < 0.001$ ) and, on average, accounted for 81% of the total matings observed per replicate.

#### Dispersal

Neither male diet ( $F_{1,36} = 0.14$ ,  $P = 0.71$ ) nor trap distance from release point ( $F_{2,36} = 1.57$ ,  $P = 0.22$ ) had a significant effect on the number of

males captured in the trimedlure-baited traps (Fig. 2). The interaction between these main factors was not significant ( $F_{2,36} = 0.01$ ,  $P = 0.99$ ). The total number of males captured per replicate was  $1155.1 (\pm 232.6)$  for sugar-agar fed males compared to  $1069.4 (\pm 242.2)$  for males fed the sugar-agar gel supplemented with yeast hydrolysate (paired  $t = 0.81$ ,  $df = 6$ ,  $P = 0.45$ ). Similar proportions of males fed sugar-agar gel or sugar-agar gel plus yeast hydrolysate were captured at the release point (26% versus 28% per replicate, respectively, paired  $t = 0.79$ ,  $df = 6$ ,  $P = 0.46$ ) or 50 m (33% versus 30%, respectively, paired  $t = 0.94$ ,  $df = 6$ ,  $P = 0.38$ ) from the release point.

#### DISCUSSION

Supplementing the adult sugar-agar diet used in SIT programs with different nitrogenous materials had no obvious effect on the mating success of mass-reared, sterile *tsl* males of the Mediterranean fruit fly. Regardless of the identity or amount of the nitrogen source used, *tsl* males dis-

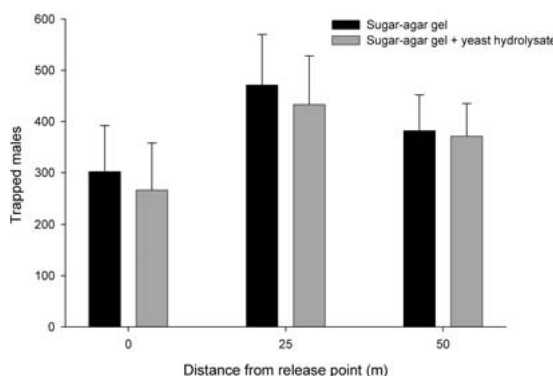


Fig. 2. Captures in trimedlure-baited traps of mass-reared, sterile *tsl* males of *Ceratitidis capitata* fed sugar-agar gel or sugar-agar plus yeast hydrolysate (10%) prior to release in a Hawaiian coffee field. Bar heights represent mean ( $\pm 1$  SE) number of males captured over a 24-h period 2 d after release at 0 (1 trap), 25 (4 traps), or 50 (8 traps) m from a central release point ( $n = 7$  replicates).

played a consistent level of mating success, achieving an average of 19% of the total matings observed per replicate. In addition, a release-recapture study revealed no difference in trap captures—either number or spatial distribution—between *tsl* males fed sugar-agar gel or sugar-agar gel supplemented with yeast hydrolysate.

As noted above, the addition of nitrogen-bearing substances (notably yeast hydrolysate) to a sugar-only or sugar-agar adult diet has invariably been shown to increase the mating success of wild males, whereas the results are mixed with mass-reared sterile males (Yuval et al. 2007). The reason(s) for this are unclear, but Yuval et al. (2007) note several possible explanations, including (i) genetic modifications resulting from bottlenecks and artificial selection associated with strain colonization and mass-rearing, (ii) the availability of carcasses or feces to sterile males, which may provide protein (even to males restricted to a sugar-only diet) and consequently obscure potential effects associated with different experimental diets, and (iii) differences in the bacterial communities in the guts of wild versus mass-reared males, which affect their ability to process and utilize different experimental diets.

Regarding the latter possibility, the addition of symbiotic gut microbes (in particular, the bacterial species *Enterobacter agglomerans* and *Klebsiella pneumoniae*) to the adult diet has been shown to improve the intestinal health, and presumably the overall physiology, of sterile male medflies (C. Lauzon, unpublished data cited in Niyazi et al. 2004). However, to our knowledge, only one study (Niyazi et al. 2004) has investigated the impact of such bacterial supplements on male mating success, and this examined only one dose combination of

microbial and nitrogenous (yeast hydrolysate) supplements to the standard sugar-agar diet. The effect of this particular combination on male mating performance was promising but not clear-cut (a significant, positive effect was observed in the laboratory but not in the field), and additional dose combinations should be evaluated.

In conclusion, it appears that any decision to use nitrogen-containing dietary supplements in pre-release diets hinges largely on the demonstration of enhanced male mating success, as there appears to be little, if any, effect of such supplements on the longevity of sterile male medflies, another parameter central to the success of SIT programs. For example, Barry et al. (2007) found no difference in the longevity of sterile males provided a pre-release diet (first 2 d of adult life) containing sugar and hydrolyzed yeast versus a pre-release diet of sugar only (this finding was independent of the composition of the post-release diet offered). Likewise, Shelly & McInnis (2003) maintained sterile males on a sugar plus yeast hydrolysate diet or a sugar-only diet, released males in field cages (over rooted trees with or without fruits), and found no diet-related difference in survival over 4-d intervals. Finally, working with large cohorts of flies in a mass-rearing facility, Muller et al. (1997) maintained sterile males on the same diet over their entire lifetime and found no difference in longevity between individuals given a sugar-only diet and those given a sugar plus hydrolyzed yeast diet. There is evidence that inclusion of protein in the post-release diet (>2-3 d of age) enhances male survival (Maor et al. 2004), but this benefit is apparently conferred to all released males independent of their pre-release diet (Barry et al. 2007).

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