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Authors: Shelly, Todd E., Nishimoto, Jon I., and Edu, James

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INFLUENCE OF METHOPRENE ON THE AGE-RELATED MATING PROPENSITY OF MALES OF THE ORIENTAL FRUIT FLY AND THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHROTIDAE)

TODD E. SHELLY, JON I. NISHIMOTO AND JAMES EDU
USDA-APHIS, 41-650 Ahiki Street, Waimanalo, Hawaii 96795

ABSTRACT

The application of juvenile hormone (or chemical analogs, such as methoprene) to newly emerged adult male fruit flies (Diptera: Tephritidae) represents a promising method to improve the efficiency of the Sterile Insect Technique against economically important species. This procedure has been shown to accelerate male sexual maturity in species with a long pre-copulatory period, and could allow for release of sterile males at younger ages and a greater release rate of sterile males overall. Topical application of methoprene has been shown to enhance male mating competitiveness. The present study investigated the effect of methoprene on maturation speed in males of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (a 'slow' maturing species) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (a 'fast' maturing species). For both species, newly emerged males were treated with acetone containing methoprene (treated) or acetone alone (control). The mating propensity of males was then monitored in non-competitive environments with mature females. Contrary to other studies, we found no evidence that methoprene accelerated male sexual activity in either a wild-like or mass-reared strain of *B. dorsalis* or a mass-reared (genetic sexing strain) of *C. capitata*. Possible explanations for these results are discussed.

Key Words: *Bactrocera dorsalis*, *Ceratitis capitata*, Sterile Insect Technique, hormone therapy, Tephritidae, methoprene

RESUMEN

La aplicación de hormonas juveniles (o análogos químicos, como metoprena) a machos adultos de moscas de la fruta (Diptera: Tephritidae) recién emergidos representa un método prometedor para mejorar la eficiencia de la técnica del insecto estéril (TIE) contra especies de importancia económica. Este procedimiento ha de mostrado que se puede acelerar el tiempo de la madurez sexual de machos en especies con un periodo largo de pre-copulación, y puede permitir la liberación de machos estériles más jóvenes e incrementar el número de machos estériles liberados. Se ha mostrado que aplicaciones de contacto de metoprena mejora la competitividad reproductiva de machos. Este estudio investiga el efecto de metoprena sobre la velocidad de madurez de los machos de la mosca oriental de la fruta, *Bactrocera dorsalis* (Hendel) (una especie que madura lentamente) y la mosca mediterránea de la fruta (*Ceratitis capitata* Wiedemann), (una especie que madura rápidamente). Por ambas especies, machos recién emergidos fueron tratados con acetona que contiene metoprena (el grupo tratado) o solo acetona (el grupo control). Se monitoreó la propensión de los machos para aparearse en ambientes no-competidos con hembras maduras. Al contrario de otros estudios, no encontramos ninguna evidencia que metoprena acelera la actividad sexual de machos en cepas de *B. dorsalis* salvajes o criados masivamente o en una cepa de *C. capitata* que separaron los sexos genéticamente criados masivamente. Se discuten las posibles causas de estos resultados.

The Sterile Insect Technique (SIT) is widely used to suppress or eradicate fruit fly pests, in particular the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Hendrichs et al. 2002; Klassen 2005; Enkerlin 2005). The process involves the mass production of the target species and the subsequent release of irradiated (sterile) individuals in the environment to achieve matings between sterile males and wild females, which yield infertile eggs and thus depress the reproductive potential of the pest population. Ultimately, the chief goal of the SIT is to produce maximally competitive sterile males at the lowest cost

over the shortest time interval. Because the large-scale production and release of insects are inherently expensive, there is a persistent need to evaluate these processes and reduce associated costs.

In recent years, many studies have been conducted to assess the impact of adult diet (Aluja et al. 2001; Shelly et al. 2005; Barry et al. 2007; Yuval et al. 2007) and adult olfactory environment (Shelly et al. 2007) on the field performance of sterile fruit fly males. Another promising avenue of investigation involves the application of juvenile hormone (or synthetic analogs) to

newly emerged males. This procedure may result in 2 significant benefits to SIT programs. First, Teal et al. (2000) demonstrated that topical application of juvenile hormone (or the synthetic mimics methoprene and fenoxycarb) to young adult males of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), dramatically accelerated sexual maturation. For example, the mean age at which all males in a replicate mated was 7 d for control males compared to 4 d for hormone-treated males. Similar trends have also been reported for males of the West Indian fruit fly, *A. obliqua* (Macquart) and the Mexican fruit fly, *A. ludens* (Loew) (Teal et al. 2007). Particularly for species, like *Anastrepha*, with relatively long pre-maturation intervals of 1-3 weeks depending on strain and species (Aluja 1994), increased rate of sexual development allows for the earlier release of sterile males, which, in turn, promotes a greater release rate of sterile males into the environment. Methoprene treatment may be less effective on fruit fly species with shorter pre-maturation intervals, and Faria et al. (2008) found no methoprene-mediated effect on sexual development in mass-reared, male Mediterranean fruit flies, whose normal pre-copulatory period was only 2-4 d.

In addition, treating young males with methoprene may enhance their copulatory success. Working with *A. suspensa*, Pereira (2005) found that protein-fed males treated with methoprene achieved significantly more matings than sexually mature, protein-fed males that had not received methoprene. However, a comparable study on *C. capitata* yielded inconsistent results. Comparing mating success among 4 treatment groups (methoprene treated or untreated and protein-fed or deprived), Faria et al. (2008) found no effect of methoprene in field cage trials (regardless of diet regime) and in laboratory trials found a significant effect of methoprene among protein-deprived males but not protein-fed males.

The present study investigated the effect of methoprene application on sexual maturation in males of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) and the Mediterranean fruit fly. In *Bactrocera* species, males of wild populations have lengthy pre-copulatory periods of 2-4 weeks (Yang et al. 1994; Wee & Tan 2000), while males of mass-reared strains mature in approximately 6-8 d (Vargas et al. 1984). Thus, the pre-maturation interval in *Bactrocera* species is comparable to that of many *Anastrepha* species, and we anticipated that methoprene would effectively accelerate sexual maturation in *B. dorsalis*, as reported for the melon fly, *B. cucurbitae* (Coquillett) (Haq et al. 2008). The work presented here on *C. capitata* was undertaken to confirm the results of Faria et al. (2008), i.e., the absence in this species of a methoprene effect on male sexual maturation.

MATERIALS AND METHODS

Study Insects

The majority of tests with *B. dorsalis* involved flies from a laboratory ("wild-like") colony started with 300-500 adults reared from mangos (*Mangifera indica* L.) collected in Waimanalo, Oahu. The colony was maintained in a screen cage (l:w:h, 1.2 × 0.6 × 0.6 m) and provided a mixture (3:1 wt:wt) of sugar (sucrose) and enzymatic yeast hydrolysate. Water was supplied *ad libitum*, and papayas (*Carica papaya* L.) were introduced for oviposition. Infested papayas were held over vermiculite, and the pupae were sifted from vermiculite 16-18 d later. Adults used in the mating trials were separated by sex within 24 h of eclosion and held in screen-covered, plastic buckets (5-L volume; 100-125 individuals per bucket) with ample food (the sugar-yeast hydrolysate mixture) 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 study, these wild-like flies were 4-5 generations removed from the wild.

As shown below, tests with the wild-like strain of *B. dorsalis* failed to show an effect of methoprene. Consequently, we performed a second set of tests using *B. dorsalis* from a mass-reared strain that had been maintained by USDA-ARS for over 20 years (D. McInnis, personal communication). Females of this strain oviposited in perforated tubes, and eggs were placed on an artificial diet (Tanaka et al. 1969) for larval development. Adults were handled in the same manner described above and held under the same environmental conditions.

For medfly, experiments were performed with mass-reared males and wild-like females. Mass-reared males were from a *tsl* (temperature sensitive lethal) genetic sexing system (Vienna-7/To1-99) reared by the California Department of Food and Agriculture's (CDFA) Hawaii Fruit Fly Rearing Facility, Waimanalo, Oahu. In rearing this strain, eggs are exposed to high temperature, which selectively kills female embryos and allows production and release of males only (Franz et al. 1994). Undyed pupae were obtained 2 d before eclosion after irradiation in air at 150 Gy of gamma radiation with a ¹³⁷Cs source.

Female medflies were from a laboratory colony started with 400-600 adults reared from infested coffee berries, *Coffea arabica* L., collected near Haleiwa, Oahu. The colony was maintained in screen cages and provided with ample food (sugar-yeast hydrolysate mixture), water, and oviposition substrate (perforated plastic vials containing small sponges soaked in lemon juice). Eggs were placed on standard larval medium (Tanaka et al. 1969) in plastic containers over vermiculite for pupation. Adults were separated by

sex within 2 d of eclosion, well before reaching sexual maturity at 7-9 d of age. Prior to testing, the adult mass-reared male and wild-like female medflies were maintained under the conditions described above for *B. dorsalis*. When used in the present study, the wild-like *C. capitata* females were 5-6 generations removed from the wild.

Chemical Treatments

For both *B. dorsalis* and *C. capitata*, we followed the methods of Teal et al. (2000) and Pereira (2005), with 1 exception noted below. For treated males, methoprene was applied topically at a dose of 5 μg in 1 μL of acetone per male. For control males, 1 μL of acetone alone was applied per male. For both male groups, the procedure was conducted within 24 h of adult emergence. Males were immobilized by chilling (5-7°C for 5-10 min), and the chemical was applied via pipette onto the dorsal surface of the thorax. Because the aforementioned authors did not anesthetize the males in their work, we assessed the possible effect of chilling on the chemical treatment by performing additional mating trials for *B. dorsalis* (wild-like strain) in which males were immobilized in a net bag (i.e., without chilling) and the chemicals were applied directly through the netting. Based on data obtained with chilled males (see below), non-chilled males were tested only at ages of 10 and 12 d. The effect of chilling was not investigated in the mass-reared males of *B. dorsalis* or *C. capitata*.

Mating Propensity

Mating propensity was monitored in laboratory cages for both study species. For the wild-like strain of *B. dorsalis*, we placed 15 males of a given age and treatment and 20 mature females (18-25 d old) in plexiglas cages (30 \times 30 \times 40 cm, with a screened opening on the top and a sleeve-covered opening on 1 side). Males were tested at 2-d age intervals between the ages of 2-18 d (i.e., when 2, 4, 6 . . . 18 d old). Because mating occurs at dusk in this species (Arakaki et al. 1984), the cages were set up in mid-afternoon (1400-1600 h), and the room lights were extinguished (natural light entered through west-facing windows). Cages were checked 3-4 h after sunset, and the number of mating pairs was recorded for individual cages. On a given test day, we set up equal numbers of cages with treated or control males of a particular age, with 6, 8, or 10 total cages established per age group per day and 1-3 different age groups tested on any given day. Fifteen cages (replicates) were run in total for treated and control males in each age group. In tests involving non-chilled males of *B. dorsalis*, 10 cages (5 treated, 5 control) were established per age group per day on each of 2 d, yielding 10 total cages per treatment group per age category.

Tests involving the mass-reared strain of *B. dorsalis* were conducted in the same manner, except that only 1 age group of males (5 d old) was tested. Initially, to describe the age-dependent mating activity of the mass-reared strain (independent of any chemical treatment), we recorded mating pairs in cages containing 15 untreated males of a given age (i.e., when 2, 3, 4 . . . 12 d old, respectively) and 20 sexually mature females (>14 d old). Fifteen cages were run in total for each 1 d age group, with 1-5 age groups tested per day and 3-5 cages tested per age group per day. As shown below, untreated males from the mass-reared strain first showed noticeable mating activity when 5 d old, and consequently we predicted that an effect of methoprene treatment would be detectable at this age. We tested 7 cages for both treated and control 5 d old males on each of 3 different days, yielding 21 replicates in total for each treatment group. Females used in these tests were all sexually mature (>14 d old).

For *C. capitata*, we placed 20 males of a given age and treatment and 30 females (8-12 d old) in screen cages (30 cm cubes). Males were tested at 1 d age intervals between the ages of 2-6 d. Mating occurs primarily in the morning in natural populations in Hawaii (Shelly et al. 1994), consequently the cages were set up in the morning (0700-0800 h). Cages were checked periodically for 4 h, and mating pairs were removed by gently coaxing them into vials. On a given day, we set up 5 cages with treated males and 5 cages with control males of the same age. Males of the same age were tested over 5 days, yielding a total of 25 cages (replicates) per treatment group.

Statistical Analysis

For both the wild-like strain of *B. dorsalis* and *C. capitata*, the effects of male age and chemical treatment were analyzed by a Friedman's test, a 2-way ANOVA by ranks, because assumptions regarding normality and homoscedasticity of the data were not met. In our analyses, male ages were blocks, and the presence or absence of methoprene in the acetone applied to the males were treatments. For each species, the test was performed with mean numbers of matings for the different male age-chemical treatment combinations (following Daniel 1990; test statistic χ^2 with $df = 1$). Thus, in our case, the Friedman test (i) ranked the (mean) number of matings obtained by treated and control males, respectively, within each age group and then (ii) compared the summed ranks across all age intervals (thus removing the effect of the blocking variable, i.e., male age).

For trials involving non-chilled *B. dorsalis* males from the wild-like strain, pair wise comparisons between the number of matings observed for treated and control males were made with the

Mann-Whitney test (test statistic T) for each of the 2 age groups considered (i.e., 10 and 12 d old), where $n_1 = n_2 = 10$ in both cases. For each of these age groups, data gathered on all 4 sets of males (chilled or non-chilled and treated or control) were compared by a Kruskal-Wallis test, where $df = 3$ in both cases (test statistic H).

For trials involving the 5 d old males from the mass-reared strain of *B. dorsalis*, pair wise comparisons between the number of matings observed for treated and control males were made by the Mann-Whitney test, where $n_1 = n_2 = 21$.

RESULTS

For the wild-like strain of *B. dorsalis*, we found no significant effect of methoprene treatment on mating propensity ($\chi^2 = 1.70$, $P = 0.21$, Fig. 1). Over both treatment groups, male mating frequency increased dramatically between 10-16 d of age and then appeared to level off between 16-18 d of age (Fig. 1).

Data gathered for non-chilled males from the wild-like strain of *B. dorsalis* similarly revealed no effect of methoprene on male mating activity. For 10-d-old, non-chilled males, individuals treated with methoprene obtained an average of 1.9 (± 0.2) matings per replicate (13% of males) compared to 1.7 (± 0.2) (11% of males) for control males ($T = 98.5$, $P = 0.65$). Likewise, for 12 d, old non-chilled males, individuals treated with methoprene obtained an average of 2.9 (± 0.3) matings per replicate (19% of males) compared to 2.6 (± 0.4) (17% of males) for control males ($T = 114.5$, $P = 0.49$). Within each of these age groups, there were no significant differences in mating propen-

sity among chilled/treated, chilled/control, non-chilled/treated, and non-chilled/control males (10 d: $H = 0.53$, $P = 0.91$; 12 d: $H = 0.25$, $P = 0.97$).

The mating activity of untreated males from the mass-reared *B. dorsalis* strain was nil at 2 or 3 d, very low at 4 d, old, but then increased rapidly between 5-9 d, after which it appeared to level off (Fig. 2). Based on these data, we selected 5 d old males to test for a possible effect of methoprene treatment on mating frequency. Among 5-d-old males, individuals treated with methoprene (when < 1 d old) obtained an average of 2.5 (± 0.3) matings per replicate (17% of males) compared to 2.1 (± 0.3) (14% of males) for control males ($T = 476.5$, $P = 0.54$).

As with *B. dorsalis*, methoprene had no apparent effect on the mating activity of *C. capitata* males ($\chi^2 = 1.80$, $P = 0.19$, Fig. 3). For both treatment groups, male mating frequency increased noticeably between the ages of 2-4 d but was similar for the ages of 4-6 d old.

DISCUSSION

In the present study, topical application of the juvenile hormone mimic methoprene to newly emerged adult males produced no significant acceleration of sexual maturation in either *B. dorsalis* or *C. capitata*. This result was obtained for 2 strains of *B. dorsalis*, 1 wild-like and the other mass-reared, and for 1 mass-reared strain of *C. capitata*.

The absence of a methoprene effect on maturation in mass-reared *C. capitata* was consistent with the study of Faria et al. (2008) and the notion that the already short pre-copulatory period (2-4

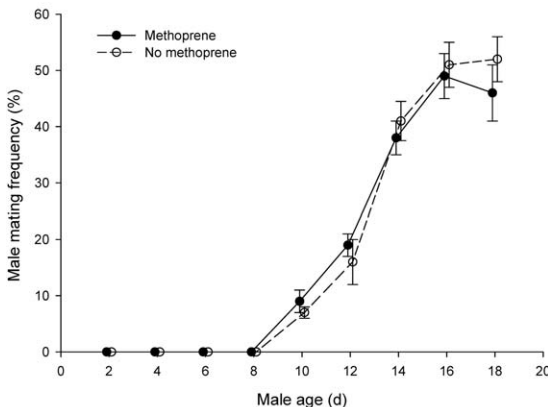


Fig. 1. Mating frequency of *Bactrocera dorsalis* males from a wild-like strain treated with acetone containing or lacking methoprene. Values along ordinate represent the mean (± 1 SE) proportion of males mating per cage (replicate) at a given age interval. Fifteen males were observed per cage, and 15 cages were run per treatment group per age group.

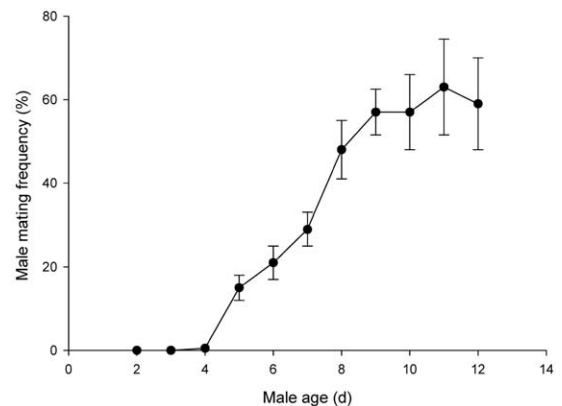


Fig. 2. Mating frequency of untreated (no chemical treatment) *Bactrocera dorsalis* males from a mass-reared strain. Values along ordinate represent the mean (± 1 SE) proportion of males mating per cage (replicate) at a given age interval. Fifteen males were observed per cage, and 15 cages were run per treatment group per age group.

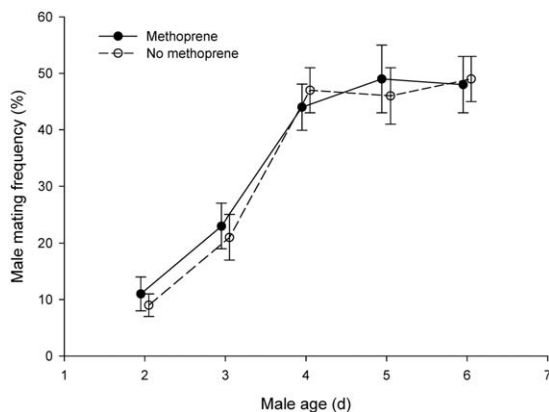


Fig. 3. Mating frequency of *Ceratitidis capitata* males from a genetic sexing strain treated with acetone containing or lacking methoprene. Values along ordinate represent the mean (± 1 SE) proportion of males mating per replicate at a given age interval. Twenty males were observed per cage, and 25 cages were run per age group.

d) lessens the opportunity for additional developmental acceleration via methoprene application. However, our failure to detect a methoprene effect in *B. dorsalis* was unexpected, because males of this species have a relatively long maturation interval and all previous studies (Teal et al. 2000, 2007; Pereira 2005) of *Anastrepha* species with a similar life history have demonstrated a pronounced increase in male sexual development following methoprene treatment. In addition, Haq (2008) reported that methoprene significantly shortened the male pre-copulatory interval in another *Bactrocera* species, *B. cucurbitae*.

There may be 2 main reasons to account for the absence of a methoprene effect in *B. dorsalis*. First, the effect of methoprene treatment of 5 μ g methoprene in 1 μ L of acetone per male (Teal et al. 2000) on the rate of male sexual maturation may vary among tephritid species and exogenous application of juvenile hormone or chemical mimics may have different effects in different species. For example, among aphids, application of juvenile hormone has been shown to promote brachyptery in some species but not in others (Zera & Denno 1997). Likewise, the application of juvenile hormone (JH III) to diapausing females was found to promote the development of vitellogenic (yolky) oocytes in one species of heteropteran (Adams et al. 2002) but not in another (Shinoda et al. 1996). A variable effect of juvenile hormone treatment has even been reported among populations within a single species: application of an intermediate dose of juvenile hormone to male larvae of a US population of the beetle *Onthophagus taurus* Schreber resulted in the production of horned adults, whereas the same dose applied to male larvae of an Australian population of the same

species resulted exclusively in horn-less adults (Moczek & Nijhout 2002), but see Zera (2007) for an alternate explanation. Thus, the demonstration of a methoprene effect on male sexual maturation in one species of *Bactrocera*, namely *B. cucurbitae* (Haq et al. 2008), does not imply that such an effect exists in all *Bactrocera* species.

Second, the absence of a methoprene effect in *B. dorsalis* might have been an inadvertent consequence of our experimental protocol. As noted, unlike previous studies, we anaesthetized *B. dorsalis* males via chilling. However, this procedural difference appears inconsequential, because supplementary tests with non-chilled males likewise failed to demonstrate a methoprene effect. Alternatively, although a methoprene effect has been demonstrated in *Anastrepha* species in small laboratory cages (Teal et al. 2000; Pereira 2005), it is possible that the use of such cages for *B. dorsalis* somehow obscured this effect. For example, if mating activities (e.g., pheromone release) of sexually mature males are inhibited in laboratory cages, then differences in mating "drive" between methoprene-treated and control males may be minimized and undetectable. However, the observation that age-related mating frequency curves were nearly identical between methoprene-treated and control *B. dorsalis* males suggests the unlikely scenario that a cage effect, if existing, was so severe that it completely eliminated behavioral differences between treated and control males. Still, as Haq's (2008) study of *B. cucurbitae* was performed in field cages, the possibility exists that a significant methoprene effect might yet be detected for *B. dorsalis* if tested under more natural conditions.

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