Ability of Sterile Males to Inhibit Female Remating in the Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)

Todd Shelly

USDA-APHIS, 41-650 Ahiki Street, Waimanalo, HI 96795. todd.e.shelly@usda.gov

Abstract. The Sterile Insect Technique (SIT) is often used to suppress or eradicate populations of invading pest species of true fruit flies (Tephritidae). The success of the SIT depends largely on the ability of mass-reared, sterile males to compete successfully against wild males to obtain copulations with wild females. In addition, as females are often polyandrous, sterile males should inhibit female remating to a degree similar to that effected by wild males. The objective of this study was to determine whether sterile males of a genetic sexing strain of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) inhibited the remating propensity of wild females to the same degree as wild males. Females were first mated with either a sterile male from the laboratory strain or a fertile wild male. Mated females were then held 1, 10, or 20 d, then offered wild males for mating, and the incidence of rematings was scored. For each time interval, remating level was higher for females first mated to sterile, laboratory males than fertile, wild males. In addition, independent of the identity of the first male mating partner, the incidence of female remating increased with time elapsed since the initial mating. Results are compared with similar studies on other pest tephritid species.

The genus *Bactrocera* Macquart (Diptera: Tephritidae) contains approximately 450 described species most of which are native to tropical Asia, the South Pacific, and Australia (Doorenweerd et al. 2018). Owing to increased international trade and tourism, however, various species have been introduced to many other parts of the world, including sub-Saharan Africa, Oceania and Hawaii, and South America (Clarke et al. 2005). Over 50 species in this genus are considered to be serious economic pests (Vargas et al. 2015), which collectively infest a broad range of fruits, including guava, apple, peach, mango, and papaya among others (White and Elson-Harris 1992, Clarke et al. 2005). Commercial losses to growers derive from direct damage to fruit, which reduces export volume, and quarantine restrictions, which limit trading opportunities.

Within the genus, the highly polyphagous oriental fruit fly, *Bactrocera dorsalis* (Hendel), is considered the most notorious horticultural pest. Females of this species lay eggs in, and larvae subsequently feed upon, approximately 490 hosts (USDA COFFHI 2020). This broad host range, along with high fecundity (Yang et al. 1994), high dispersal ability (Froerer et al. 2010), and competitive superiority over resident tephritid species (Duyck et al. 2004), result in high invasive ability (Aketarawong et al. 2014), and *B. dorsalis* has now been reported from over 65 countries worldwide (CABI 2019). The threat posed to agriculture has prompted many countries to operate continuous, large-scale surveillance programs to detect infestations and, upon detection, to implement costly suppression or eradication efforts (e.g., Steck et al. 2019).

Several methods, implemented either singly or in concert, have been used to control outbreaks of *B. dorsalis*, including protein bait sprays, soil drenches, the Male Annihilation Technique (MAT), parasitoid release, and the Sterile Insect Technique (SIT) (Vargas et al. 2015). MAT, which involves the field deployment of the powerful male lure methyl eugenol (4-allyl-1, 2-dimethoxybenzene-carboxylate) to greatly reduce or eliminate the male population, has been the most successful stand-alone eradication tool (Vargas et al. 2014). Because of the success of MAT, SIT has been used as less frequently against *B. dorsalis* than against other tephritid pests, such as the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), or various *Anastrepha* species (Enkerlin 2005).

When implemented, however, SIT has been effective in eradicating small populations of *B. dorsalis*. SIT involves the release of large numbers of sterile individuals (obtained via pupal irradiation) of the target species, with the aim of generating sterile male by wild female crosses, which result in inviable progeny and the subsequent decline of the pest population (Knipling 1955). When employed against *B. dorsalis*, SIT is often conducted following a program of MAT, which serves to reduce the population to allow high overflooding ratios (sterile male:wild male ratios) and thereby increase the effectiveness of SIT. This MAT-then-SIT strategy has resulted in the successful eradication of *B. dorsalis* in several instances (Habu et al. 1984, Shiga 1989, Steiner et al. 1970). In conjunction with orchard sanitation and protein bait spraying, SIT led to substantial reduction of *B. dorsalis* in mango-growing areas in Thailand (Orankanok et al. 2007). Although not frequently used as a stand-alone tactic, SIT alone led to the eradication of a small population of *B. dorsalis* on Guam (Steiner et al. 1970).

The success of the SIT depends largely on the ability of mass-reared, sterile males to compete successfully against wild males to obtain copulations with wild females (Calkins 1984). Unlike the Mediterranean fruit fly, studies consistently show that mass-reared, sterile males of various *Bactrocera* species are equivalent, or even superior, sexual competitors relative to wild males (Shelly and McInnis 2016), a finding that applies to *B. dorsalis* as well (Shelly 1995, Shelly et al. 1996, 2000, Orankanok et al. 2013). In addition to high mating competitiveness, sterile males should inhibit female remating to a degree similar to that effected by wild males. Female tephritids are, in general, polyandrous but exhibit reduced receptivity for a certain time (termed the refractory period) after mating (Abraham et al. 2016). If, in the worst case, a sterile male mates with a virgin wild female but does not reduce her mating propensity, the female may subsequently mate with a wild male, thus rendering the initial mating (with the sterile male) inconsequential as a means of population reduction. Surprisingly, relatively few studies have mated virgin wild females with either wild males or mass-reared, sterile males and then measured the remating tendency of these females when subsequently offered wild males, and these have focused primarily on the Mediterranean fruit fly (Hendrichs et al. 1996, McInnis et al. 2002, Vera et al. 2003, Mossinson and Yuval 2003) or the Mexican fruit fly, *Anastrepha ludens* (Loew) (Meza et al. 2014, Arredondo et al. 2017).

Focusing on *B. dorsalis*, the goal of this study was to compare the abilities of sterile males from a genetic sexing strain and fertile wild males to inhibit female remating when females were offered a second mating opportunity with a wild male. Moreover, female remating tendency was measured at varying intervals after the initial mating with a sterile or wild male. Results are compared with those recently obtained for the melon fly, *Zeugodacus*

cucurbitae (Coquillett), using the same experimental design (Shelly 2019).

Materials and Methods

Insects. Wild flies were obtained from a recently established laboratory colony started with approximately 2,000 adults that emerged from guava fruits (*Psidium guajava* L.) collected near Keaau, Hawaii island. Eggs were gathered using artificial oviposition devices (perforated plastic vials containing a small guava juice-soaked sponge) and placed on standard larval medium (Tanaka et al. 1969) held in containers above a layer of vermiculite for pupation. Adult flies were separated by sex within 2–3 d of emergence and maintained on standard adult diet (3:1 mixture of sugar and protein hydrolysate). When first mated, wild flies were 18–22 d old. When tested, flies from this colony were 4–6 generations removed from the wild.

The genetic sexing strain (labelled DTWP) was developed approximately 25 years ago (McCombs and Saul 1995) and has since been reared continuously at USDA-ARS facilities in Hawaii. In this strain, a so-called first generation or pupal color sexing strain, inheritance of pupal color is linked to sex determination via a Y-autosomal translocation, such that males have the wild type brown pupae and females have mutant white pupae (Meza et al. 2018). This color difference allows for separation of the sexes and all-male releases, which are considered more effective in SIT than traditional bisexual releases (Hendrichs et al. 1995). The DTWP strain was reared in the same manner described above for the wild colony. To obtain sterile males for the matings, pupae were irradiated at 100 Gy 2 d before eclosion under hypoxia with a ^{137}Cs irradiator at the USDA-APHIS Irradiation Facility, Waimanalo, HI. Adult DTWP males were collected within 2 d of emergence and mated when 12–15 d old (DTWP males

mature more rapidly than wild males, Shelly unpublished data). DTWP females were not used in this study. Rearing of both wild and DTWP strains and mating tests were conducted at 24–27°C, 50–70% RH, and a 13:11 h (L:D) photoperiod with natural and artificial light.

Mating trials. To obtain mated females, I placed a virgin wild female and a virgin male in each of 50–80 numbered jars (3.75 L volume) on a given date. Approximately half of the jars contained wild males, while the other half contained sterile DTWP males. The jars were transparent plastic, and a cloth sleeve covered the mouth to allow ventilation as well as access to the flies. Pairs were established 2–3 h before dusk, the period of sexual activity in *B. dorsalis* (Arakaki et al. 1984). At sunset, jars containing mating pairs were recorded, and it was assumed that, as characteristic of the species (Arakaki et al. 1984), pairs remained coupled until sunrise. The following morning, mated females were placed in screen cages (30 cm cubes; 20-30 females per cage) according to the identity of their mating partner; food and water were supplied.

Mated females were offered an opportunity to remate with a wild male at 1, 10, or 20 d after their initial mating. For these remating trials, 10 females mated to either a wild or sterile DTWP male were placed with 10 virgin wild males in plexiglass cages (40 x 30 x 30 cm, l:w:h) 2–3 h before sunset, and total matings were scored per cage shortly after sunset. Six cages were observed per night, one for each of the three remating intervals for females mated initially to a wild or sterile DTWP male. Data were collected on eight separate nights. For females tested at 10 or 20 d, egging devices were placed in the holding cage for 6–8 h on alternate days. Allowing oviposition likely increased the incidence of female remating (Sivinski and Heath 1988, Landolt 1994 but see

Landeta-Escamilla et al. 2016), but this was considered a more realistic scenario than complete deprivation of egg-laying opportunities.

Analysis. Raw counts of female rematings met the parametric assumptions of normality and equal variances, consequently data were analyzed using a 2-way ANOVA with male type and time elapsed since the initial mating as the main effects. The Holms-Šidák multiple comparisons test was used to identify pair wise differences. Statistical analyses were performed using SigmaPlot v. 11 (Systat Software, San Jose, CA).

Results

Both male type ($F_{1,42} = 19.5$, $P < 0.001$) and time interval since initial mating ($F_{2,42} = 13.6$, $P < 0.001$) had significant effects on female remating tendency (Fig. 1). The interaction between these variables was not significant ($F_{2,42} = 0.10$, $P = 0.91$). Across all time intervals, female remating was found to be significantly higher following an initial mating with a sterile male than a wild male ($t = 4.4$, $P < 0.001$; Holms-Šidák test). The same post hoc test revealed that, independent of the identity (sterile or wild) of the first mating partner, female remating levels differed significantly between 1 and 20 d ($t = 5.0$) and 10 and 20 d ($t = 3.7$) after the initial mating, respectively, ($P < 0.001$ in both cases) but not between 1 and 10 d ($t = 1.3$, $P = 0.21$).

Discussion

The present study reveals two main findings. First, the remating tendency of once-mated *B. dorsalis* females generally increased with time elapsed from their initial mating regardless of whether the first mate was a wild, fertile or a lab-reared, sterile male. This same trend was observed for *Z. cucurbitae* females when tested under the same experimental design (Shelly 2019) and is consistent with

data from other tephritids that showed increased female remating when host fruits are available (vs. fruit deprivation; Sivinski and Heath 1988, Landolt 1994). This pattern has been ascribed to sperm depletion, but two lines of evidence suggest that the amount of sperm stored does not—by itself—explain female remating tendency in *B. dorsalis*. First, in other studies on wild *B. dorsalis*, where female remating was scored weekly over 6–8 weeks and host fruit was available periodically for oviposition, the incidence of female remating did not increase over time (as expected if sperm depletion were important) but instead was relatively constant (Shelly 2000, Shelly and Edu 2008). Second, in the closely related species *Z. cucurbitae*, sperm-depleted, irradiated males inhibited female remating to the same degree as fertile males, indicating that sperm supply in the female does not influence female remating in that species and possibly *B. dorsalis* (Kuba and Itô 1993). Indeed, data from the Queensland fruit fly, *B. tryoni* (Froggatt), also show that female remating is modulated, not by sperm store, but by accessory gland products transferred in the ejaculate (Harmer et al. 2006, Radhakrishnan and Taylor 2007, Radhakrishnan et al. 2009). Clearly, a more complete understanding of remating by *B. dorsalis* females awaits comparable research on underlying physiological mechanisms.

The second main finding of the present study was the reduced ability of sterile males from the DTWP genetic sexing strain to inhibit female remating relative to that observed for fertile wild males. This difference was evident independently of the time elapsed since the female's initial mating. This same result was obtained for *Z. cucurbitae* under nearly identical experimental conditions (Shelly 2019). In an earlier study, Kuba and Itô (1993) found that wild *Z. cucurbitae* females
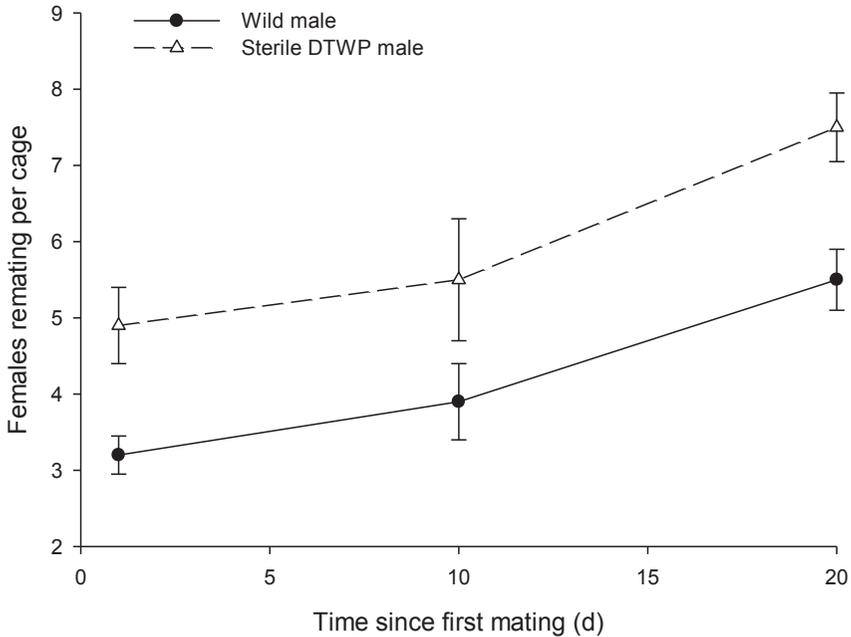
Figure 1. Numbers of rematings observed per cage for females first mated to fertile wild or sterile DTWP males at 3 intervals after the initial mating. Each cage held 10 test females. Symbols represent mean values ± 1 SE; N = 8 in all cases.

were more likely to remate (at 3 d after the first mating) when the first mate was a fertile laboratory strain male than when the first mate was a fertile wild male, suggesting a strain difference, and not irradiation, may have accounted for the observed difference in female remating. In contrast to these results, other studies on *Bactrocera* or *Zeugodacus* species have reported no difference between sterile and fertile males in inhibiting female remating (Harmer et al. 2006, Radhakrishnan et al. 2009, Haq et al. 2013). However, these studies either used laboratory strains exclusively (i.e., no wild flies) or a different experimental design that confounds comparison with the present study. Inhibition of female remating by sterile males has been examined more intensively for the Mediterranean fruit fly than any other tephritid species. Considering only data

generated via the same experimental approach used here (i.e., wild females mated initially to sterile laboratory males or wild males and then female remating measured when offered only wild males as potential mate), wild females are generally found to remate more often following a first mating with a sterile male than with a wild male (Hendrichs et al. 1996, Vera et al. 2003, Mossinson and Yuval 2003, but see McInnis et al. 2002).

In conclusion, data presented here for the *B. dorsalis* and previously for *Z. cucurbitae* (Shelly 2019) show that sterile males from a genetic sexing strain do not inhibit female remating as effectively as wild males. As noted above, this may limit the effectiveness of SIT, because, even if such males successfully procure matings with virgin females, these females may seek an additional copulation and

pair next with a wild male. Few data are available on sperm competition in *B. dorsalis*, and the results of Zhao et al. (2013) indicated considerable sperm mixing in twice-mated females. In particular, when females were mated first to a sterile male and then to a fertile male, 55% of the eggs hatched. The potential for reduced SIT effectiveness appears real given high levels of multiple mating by *Bactrocera* and *Zeugodacus* females. To my knowledge, Song et al. (2007) provide the only molecular genetic data investigating polyandry in wild-caught females, and these indicate that 23% of *B. cacuminata* (Hering) females produced offspring sired by at least two different males. Based on laboratory studies in which mates are continuously, or at least frequently, available, the proportions of females mating two or more times are 88% for *Z. cucumis* (French) (Chinajariyawong et al. 2010), 74% for *B. cacuminata* (Chinajariyawong et al. 2010), 50% for *Z. tau* (Walker) (Ooi and Wee 2016), and 14% for *B. carambolae* Drew & Hancock (Wee and Tan 2000). Comparable studies of *B. dorsalis* have generated female remating frequencies of 52% (Shelly 2000) and 77% (Wee and Tan 2000; note the species epithet *B. papayae* Drew & Hancock used in that study has recently been synonymized with *B. dorsalis*; Schutze et al. 2015). Collectively, these values are similar to those gathered for the Mediterranean fruit fly (Mossinson and Yuval 2003), suggesting that remating inhibition by released, sterile males is an important determinant of SIT's success against tephritid pests in general.

Acknowledgments

Literature Cited

Abraham, S., M. Herrera-Cruz, and **D. Pérez-Staples.** 2016. Female remating behavior in pest tephritid fruit flies and its implication for the Sterile Insect Technique, pp. 323–338. *In* B. Sabater-Muñoz, T. Vera, R. Perreira, and W. Orankanok. (eds.) Proc. 9th Intl. Symp. Fruit Flies Econ. Importance. ISBN: 978-616-358-207-2.

Akertarawong, N., C.R. Guglielmino, K. Karam, M. Falchetto, M. Manni, F. Scolari, L.M. Gomulski, G. Gasperi, and **A.R. Malacrida.** 2014. The oriental fruit fly *Bactrocera dorsalis* s.s. in East Asia: distentangling the different forces promoting the invasion and shaping the genetic make-up of populations. Genetica 142: 201–213.

Arakaki, N., H. Kuba, and **H. Soemori.** 1984. Mating behavior of the oriental fruit fly, *Dacus dorsalis* Hendel (Diptera: Tephridae). Appl. Entomol. Zool. 19: 42–51.

Arredondo, J., M.T. Tejeda, L. Ruiz, J.S. Meza, and **D. Pérez-Staples.** 2017. Timing of irradiation and male mating history effects on female remating in *Anastrepha ludens* (Diptera: Tephritidae). Fla. Entomol. 100: 566–570.

Calkins, C.O. 1984. The importance of understanding fruit fly mating behavior in sterile male release programs (Diptera, Tephritidae). Folia Entomol. Mex. 61: 205–213.

Chinajariyawong, A., R.A.I. Drew, A. Meats, S. Balagawi, and **S. Vijaysegaran.** 2010. Multiple mating by females of two *Bactrocera* species (Diptera: Tephritidae: Dacinae). Bull. Entomol. Res. 100: 325–330.

Clarke, A.R., K.F. Armstrong, A.E. Carmichael, J.R. Milne, S. Raghu, G.K. Roderick, and **D.K. Yeates.** 2005. Invasive phytophagous pests arising through a recent tropical evolutionary radiation: The *Bactrocera dorsalis* complex of fruit flies. Annu. Rev. Entomol. 50: 293–319.

Doorenweerd, C., L. Leblanc, A.L. Norrbom, M. San Jose, and **D. Rubinoff.** 2018. A global checklist of the 932 fruit fly species in the tribe Dacini (Diptera, Tephritidae). ZooKeys 730: 19–56.

Duyck, P.F., P. David, and **S. Quilici.** 2004. A review of relationships between interspecific competition and invasions in fruit flies (Diptera: Tephritidae). Ecol. Entomol. 29: 511–520.

Enkerlin, W.R. 2005. Impact of fruit fly control programmes using the Sterile Insect Technique, pp. 651–676. *In* V.A. Dyck, J. Hendrichs, and A.S. Robinson (eds.) Sterile Insect Technique: Principles and practice in area-wide integrated pest management. Springer, Dordrecht, The Netherlands.

Froerer, K.M., S.L. Peck, G.T. McQuate, R.I. Vargas, E.B. Jang, and **D.O. McInnis.** 2010. Long-distance movement of *Bactrocera dorsalis* (Diptera: Tephritidae) in Puna, Hawaii; How far can they go? Am. Entomol. 56: 88–95.

Habu, N., M. Iga, and **K. Numazawa.** 1984. An eradication program of the oriental fruit fly, *Dacus dorsalis* Hendel (Diptera: Tephritidae), in the Ogasawara (Bonin) Islands. I. Eradication field test using a sterily fly release method on small islets. Appl. Entomol. Zool. 19: 1–7.

Haq, I., M.J.B. Vreysen, A. Abd-Alla, and **J. Hendrichs.** 2013. Ability of genetic sexing strain male melon flies (Diptera: Tephritidae) to suppress wild female remating: implications for SIT. Fla. Entomol. 96: 839–849.

Harmer, A.M.T., P. Radhakrishnan, and **P.W. Taylor.** 2006. Remating inhibition in female Queensland fruit flies: effects and correlates of sperm storage. J. Insect Physiol. 52: 179–186.

Hendrichs, J., B. Katsoyannos, K. Gaggl, and **V. Wornoayporn.** 1996. Competitive behavior of males of Mediterranean fruit fly, *Ceratitis capitata*, genetic sexing strain Vienna-42, pp. 405–414. *In* B.A. McPheron and G.J. Steck (eds.) Fruit fly pests: A world assessment of their biology and management. St. Lucie Press, Delray Beach, FL.

Hendrichs, J., G. Franz, and **P. Rendon.** 1995. Increased effectiveness and applicability of the Sterile Insect Technique through male-only releases for control of Mediterranean fruit flies during fruiting seasons. J. Appl. Entomol. 119: 371–377.

Knipling, E.F. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. J. Econ. Entomol. 48: 59–62.

Kuba, H., and **Y. Itô.** 1993. Remating inhibition in the melon fly, *Bactrocera* (= *Dacus*) *cucurbitae* (Diptera: Tephritidae): copulation with sperm-less males inhibits female remating. J. Ethol. 11: 23–28.

Landeta-Escamilla, A., E. Hernández, J. Arredondo, F. Díaz-Fleischer, and **D. Pérez-Staples.** 2016. Male irradiation affects female remating behavior in *Anastrepha serpentina* (Diptera: Tephritidae). J. Insect Physiol. 85: 17–22.

Landolt, P.J. 1994. Mating frequency of the papaya fruit fly (Diptera: Tephritidae) with and without host fruit. Fla. Entomol. 77: 305–312.

McCombs, S.D., and **S.H. Saul.** 1995. Translocation-based genetic sexing system for the oriental fruit fly (Diptera: Tephritidae) based on pupal color dimorphism. Ann. Entomol. Soc. Am. 88: 695–698.

McInnis, D.O., P. Rendon, and **J. Komatsu.** 2002. Mating and remating of medflies (Diptera: Tephritidae) in Guatemala: individual fly marking in field cages. Fla. Entomol. 85: 126–137.

Meza, J.S., I. Haq, M.J.B. Vreysen, K. Bourtzis, G. A. Kyritsis, and **C. Cáceres.** 2018. Comparison of classical and transgenic genetic sexing strains of Mediterranean fruit fly (Diptera: Tephritidae) for application of the Sterile Insect Technique. PLoS ONE 13(12): e0208880.

Meza, J.S., J. Arredondo, D. Orozco, and **D. Pérez-Staples.** 2014. Disparity in sexual behavior between wild and mass-reared Mexican fruit flies. Physiol. Entomol. 39: 263–270.

Mossinson, S., and **B. Yuval.** 2003. Regulation of sexual receptivity of female Mediterranean fruit flies: old hypotheses revisited and a new synthesis proposed. J. Insect Physiol. 49: 561–567.

Ooi, Y.T., and **S.L. Wee.** 2016. Sexual maturation, mating propensity and remating incidence of *Zeugodacus tau* (Walker) (Diptera: Tephritidae). J. Asia-Pac. Entomol. 19: 451–457.

Orankanok, W., S. Chinvinijkul, A. Sawatwangkhoung, S. Pinkaew, and **S. Orankanok.** 2013. Methyl eugenol and pre-release diet improve mating performance of young *Bactrocera dorsalis* and *Bactrocera correcta*. J. Appl. Entomol. 137 (Suppl. 1): 200–209.

Orankanok, W., S. Chinvinijkul, S. Thanaphum, P. Sitilob, and **W.R. Enkerlin.** 2007. Area-wide integrated control of oriental

fruit fly *Bactrocera dorsalis* and guava fruit fly *Bactrocera correcta* in Thailand, pp. 517–526. *In* M.J.B. Vreysen, A.S. Robinson, and J. Hendrichs (eds.) Area-wide control of insect pests. Springer, Dordrecht, The Netherlands.

Radhakrishnan, P., and **P.W. Taylor**. 2007. Seminal fluids mediate sexual inhibition and short copula duration in mated female Queensland fruit flies. J. Insect Physiol. 53: 741–745.

Radhakrishnan, P., D. Pérez-Staples, C.W. Weldon, and **P.W. Taylor.** 2009. Multiple mating and sperm depletion in male Queensland fruit flies: effects on female remating behaviour. Anim. Behav. 78: 839–846.

Schutze, M.K., N. Aketarawong, W. Amornsak, K.F. Armstrong, et al. 2015. Synonymization of key pest species within the *Bactrocera dorsalis* species complex (Diptera: Tephritidae): Taxonomic changes based on a review of 20 years of integrative morphological, molecular, cytogenetic, behavioural, and chemoecological data. Syst. Entomol. 40: 456–471.

Shelly, T.E. 2019. Ability of sterile males to inhibit female remating in the melon fly *Zeugodacus cucurbitae* (Diptera: Tephritidae). Fla. Entomol. 102: 278–280.

Shelly, T.E. 2000. Fecundity of female oriental fruit flies (Diptera: Tephritidae): effects of methyl eugenol-fed and multiple mates. Ann. Entomol. Soc. Am. 93: 559–564.

Shelly, T.E. 1995. Methyl eugenol and the mating competitiveness of irradiated male *Bactrocera dorsalis* (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 88: 883–886.

Shelly, T.E., and **D.O. McInnis.** 2016. Sterile Insect Technique and control of tephritid fruit flies: do species with complex courtship require higher overflooding ratios. Ann. Entomol. Soc. Am. 109: 1–11.

Shelly, T.E., and **J. Edu.** 2008. Do methyl eugenol-fed males of the oriental fruit fly (Diptera: Tephritidae) induce female remating? Fla. Entomol. 91: 388–393.

Shelly, T.E., S.D. McCombs, and **D.O. McInnis.** 2000. Mating competitiveness of male oriental fruit flies from a translocation strain (Diptera: Tephritidae). Environ. Entomol. 29: 1152–1156.

Shelly, T.E., S. Resilva, M. Reyes, and **H. Bignayan.** 1996. Methyl eugenol and mating competitiveness of irradiated male *Bactrocera philippinensis* (Diptera: Tephritidae). Fla. Entomol. 79: 481–488.

Shiga, M. 1989. Current programme in Japan, pp. 365–374. *In*: A.S. Robinson and G.H.S. Hooper (eds.) Fruit flies, their biology, natural enemies and control. Vol. 3B. Elsevier, Amsterdam.

Sivinski, J., and **R.R. Heath.** 1988. Effects of oviposition on remating, response to pheromones, and longevity in the female Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 81: 1021–1024.

Song, S.D., R.A.I. Drew, and **J.M. Hughes.** 2007. Multiple paternity in a natural population of a wild tobacco fly, *Bactrocera cacuminata* (Diptera: Tephritidae), assessed by microsatellite DNA markers. Mol. Ecol. 16: 2353–2361.

Steck, G.J., A.J. Fox, D. Carrillo, D. Dean, A. Roda, N.D. Epsky, and **T.R. Smith.** 2019. Oriental fruit fly eradication in Florida 2015-2016 program implementation, unique aspects, and lessons learned. Am. Entomol. 65: 108–121.

Steiner, L.F., W.G. Hart, E.J. Harris, R.T. Cunningham, K. Ohinata, and **D.C. Kamakahi.** 1970. Eradication of the oriental fruit fly from the Mariana Islands by the methods of male annihilation and sterile insect release. J. Econ. Entomol. 63: 131–135.

Tanaka, N., L.F. Steiner, K. Ohinata, and **R. Okamoto.** 1969. Low-cost larval rearing medium for mass production of oriental and Mediterranean fruit flies. J. Econ. Entomol. 62: 967–968.

USDA COFFHI [Compendium of Fruit Fly Host Information] 2020. Edition 5. https://coffhi.cphst.org/.

Vargas, R.I., J.C. Piñero, and **L. Leblanc.** 2015. An overview of pest species of *Bactrocera* fruit flies (Diptera: Tephritidae) and the integration of biopesticides with other biological approaches for their management with a focus on the Pacific Region. Insects 6: 297–318.

Vargas, R.I., L. Leblanc, J.C. Piñero, and **K.M. Hoffman.** 2014. Male annihilation, past, present, and future, pp. 493–511. *In* T. Shelly, N. Epsky, E.B. Jang, J. Reyes-Flores, and R. Vargas (eds.) Trapping and

the detection, control, and regulation of tephritid fruit flies. Springer, Dordrecht, The Netherlands.

Vera, M.T., J.L. Cladera, G. Calcagno, J.C. Vilardi, D.O. McInnis, and **Field Working Group.** 2003. Remating of wild *Ceratitis capitata* (Diptera: Tephritidae) females in field cages. Ann. Entomol. Soc. Am. 96: 563–570.

Wee, S.L., and **K.H. Tan. 2000.** Sexual maturity and intraspecific mating success of two sibling species of the *Bactrocera* dorsalis complex. Entomol. Exp. Appl. 94: 133–139.

White, I.M., and **M.M. Elson-Harris.** 1992. Fruit flies of economic significance: their identification and bionomics. CABI, Wallingford, UK.

Yang, P., J.R. Carey, and **R.V. Dowell.** 1994. Temperature influences on the development and demography of *Bactrocera dorsalis* (Diptera: Tephritidae) in China. Environ. Entomol. 23: 971–974.

Zhao, J., S. Chen, Y. Deng, R. He, J. Ma, F. Liang, and **M. Hu.** 2013. Sperm precedence pattern and the effect of irradiation on male mating competition in the oriental fruit fly, *Bactrocera dorsalis*. Biohelikon Cell Bio. 2013 1:2.