

2012

## Female mating failure and the failure of 'mating' in sterile insect programs

Diana Pérez-Staples

*INBIOTECA Universidad Veracruzana*, [diperez@uv.mx](mailto:diperez@uv.mx)

Todd E. Shelly

*USDA-APHIS*, [todd.e.shelly@aphis.usda.gov](mailto:todd.e.shelly@aphis.usda.gov)

Boaz Yuval

*Hebrew University of Jerusalem*, [yuval@agri.huji.ac.il](mailto:yuval@agri.huji.ac.il)

Follow this and additional works at: <http://digitalcommons.unl.edu/usdaarsfacpub>

---

Pérez-Staples, Diana; Shelly, Todd E.; and Yuval, Boaz, "Female mating failure and the failure of 'mating' in sterile insect programs" (2012). *Publications from USDA-ARS / UNL Faculty*. 1118.

<http://digitalcommons.unl.edu/usdaarsfacpub/1118>

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Publications from USDA-ARS / UNL Faculty by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

## Female mating failure and the failure of 'mating' in sterile insect programs

Diana Pérez-Staples<sup>1\*</sup>, Todd E. Shelly<sup>2</sup> & Boaz Yuval<sup>3</sup>

<sup>1</sup>INBIOTECA Universidad Veracruzana, Av. de las Culturas Veracruzanas 101, Col. E. Zapata, Xalapa, Veracruz c.p. 91090, Mexico, <sup>2</sup>USDA-APHIS, 41-650 Ahiki Street, Waimanalo, HI 96795, USA, and <sup>3</sup>Department of Entomology The Hebrew University of Jerusalem, PO Box 12, Rehovot 76100, Israel

Accepted: 18 June 2012

**Key words:** Tephritidae, Lepidoptera, mosquitoes, weevils, leks, remating, female choice, mass-rearing, irradiation, sperm, SIT

### Abstract

The sterile insect technique (SIT) involves the inundative release of irradiated (sterile or partially sterile) insects to decrease population levels in a target pest species. The effectiveness of SIT programs depends on sterile males mating successfully and inducing reproductive failure in wild females, or in the F1 generation in the case of lepidopteran species. Thus, from the perspective of insect control, female mating failure involves mating with a mass-reared, sterilized male, which then results in female reproductive failure. Here, we review female mating failure in the context of SIT at two stages. First, at the pre-copulatory stage we consider factors that affect female mating failure with sterile males, such as differences between sterile and wild males in terms of male courtship success, male discrimination of females, pheromone production, and dispersal. We emphasize studies with some degree of ecological realism and review certain factors that can affect female sexual development and choice, such as diet, age, and sex ratio. Second, at the post-copulatory stage we consider factors that functionally result in female reproductive failure, such as ejaculate transfer and control of female remating. Sterile insect technique operations strive to incorporate methods that increase wild female mating with sterile males so that ultimately population-wide reproductive failure is achieved in the target species.

### Introduction

Variation in reproductive success is a key component of sexual selection, with the magnitude of variation considered an important determinant of the intensity of sexual selection. Mating success, a chief measure of reproductive success, is typically more variable among males, and hence males may be subject to more pervasive sexual selection pressures than females. However, as Rhainds (2010) points out, there are many examples among insect species in which females may have suboptimal, or even no, mating success because of low population density, skewed sex ratios, lack of mating opportunities, predation during the pre-sexual maturity period, and/or female aggression.

Rhainds defined female mating failure as including both females that remain virgin through their entire life span and those that mate, but remain infertile due to, for example, inadequate sperm transfer.

In the context of pest control, female mating failure is the goal for control methods that focus on reducing or preventing matings between wild individuals. One of these methods, used particularly in Lepidoptera, is mating disruption. Population levels are controlled by disrupting intersexual chemical communication through the saturation of a given area with pheromones, such that males cannot find females (Cardé & Minks, 1995). The other major approach to induce female mating failure is the Sterile Insect Technique (SIT). In SIT, the target wild females must, somewhat paradoxically, mate with the control agent (mass-reared sterile males) to achieve reproductive failure, owing to the unviability of eggs produced by such crosses. In species where pre-copulatory courtship displays are prominent, it is essential that sterile males meet

\*Correspondence: Diana Pérez-Staples, INBIOTECA, Universidad Veracruzana, Av. de las Culturas Veracruzanas 101, Col. E. Zapata, Xalapa, Veracruz c.p. 91090, Mexico. E-mail: diperez@uv.mx

behavioral criteria and are accepted as mates by wild females. Then, if females mate successfully with the control agent, this will ultimately induce reproductive failure at the post-copulatory stage as females will be rendered sterile. Thus, SIT depends on producing and releasing sterile males that can mate with and induce sterility in wild females. If females fail to mate with sterile males then the effectiveness of SIT will be reduced. From the perspective of insect SIT, the issue of female mating failure assumes the opposite focus, namely maximizing the likelihood that wild females copulate with sterile males and thereby suffer reproductive failure.

Here, we review female mating failure at the pre- and post-copulatory stages for species that are controlled using SIT. At the pre-copulatory stage, we review factors that can lead toward wild female rejection of, or lack of mating encounters with, sterile males, whereas at the post-copulatory stage we review factors that may lead to reproductive failure, such as sperm and accessory gland product transfer from sterile males to wild females, and female remating inhibition.

#### **Sterile insect technique**

The SIT is used against a number of tephritid fruit fly, mosquito, lepidopteran, and weevil pests (Vreysen et al., 2006; Hendrichs et al., 2007). The SIT consists of the mass-production, sterilization (usually by irradiation), and release of mass-reared individuals into infested areas (Knippling, 1955). For SIT to be effective, sterile males need to locate, attract, court, copulate, effectively inseminate, and inhibit wild female remating.

Due to the exigencies of colonization and mass-rearing, followed by the stress and damage incurred by sterilization, shipping, and release, SIT programs usually produce males that are of lower quality than wild males (Liimatainen et al., 1997; Cayol, 2000). At the heart of this problem is the ability of males to succeed in mating with wild females, and this is particularly relevant when the mating system is complex, as in species that form leks and where females are choosy (Lance & McInnis, 2005). In species where the mating system is simpler, mass-reared irradiated males may have greater success (Lux et al., 2002b). For example, in the tsetse fly *Glossina morsitans morsitans* (Wiedemann), females have a limited chance of rejecting males because males can intercept females passing briefly through male swarms (Wall & Langley, 1993). Similarly, assuming that the swarming habit is not lost during colonization, sterile male mosquitoes may copulate with females that approach swarms (Yuval, 2006).

#### **Pre-copulatory mating failure**

Given the importance of wild female mating failure for SIT, it is somewhat surprising that there are few studies on

female mating success from the field before SIT is implemented. An assessment of the reproductive and physiological condition of females caught in traps can shed some light on this issue, even though this does not take into account sexually mature young females captured as virgins, who would have gone on to mate and reproduce if their normal activities had not been interrupted. In general, we have little data on the extent of female mating failure in natural conditions for species in which SIT is used. For the few species where some data are available, it is clear that there are species- and population-specific differences.

Studies on wild populations of mosquitoes suggest species-specific patterns of mating success and failure, modulated within species by physiological and environmental factors. The picture emerging may be further clouded by differences in sampling methods. The following examples illustrate this point. Strikingly, in field-collected samples of *Aedes aegypti* (L.) (n = 176) and *Aedes albopictus* (Skuse) (n = 141), all dissected females were inseminated (Tripet et al., 2011). As these females were collected from two sites on seven occasions spanning 3 years, the finding suggests that mating failure in these species is rare. In a laboratory study of *Anopheles gambiae* Giles, 16% of reproductively mature females failed to mate (given one opportunity at either 1 or 3 days of age). Whereas 23% of younger females failed to mate, only 10% of the older females failed, suggesting that, given the opportunity, mating occurs eventually for females. In the same study, 25% of inseminated, blood-fed females did not oviposit, thus effectively failing to reproduce. Here too the age effect was significant as 50% of the younger vs. 25% of the older females failed to oviposit (Voordouw et al., 2008). In *A. gambiae* collected in the field in Mali, only 7% of dissected females (n = 322) were not inseminated (Tripet et al., 2003). In another study on this species, close to 50% of host-seeking females (n > 5 000) were virgins (Charlwood et al., 2003).

Field studies on tephritid fruit flies provide a similar picture, possibly with less interspecific variation and more sampling bias (e.g., only a few populations were considered for a few species). Of 47 females of the tobacco fly, *Bactrocera cacuminata* (Hering), netted around their host plant, only 46% produced progeny (Song et al., 2007). This suggests that more than half of field-collected females had failed to mate at the time they were collected. However, we do not know whether the rest of the females were not inseminated or simply failed to oviposit in the laboratory. Of 36 medfly females, *Ceratitis capitata* (Wiedemann), captured on the island of Chios, 26 (72%) went on to lay fertile eggs (Bonizzoni et al., 2002, 2006). Conversely, fewer than 30% of females collected in the field in Israel produced offspring (n = 50; Bonizzoni et al.,

2006). However, 91% of females collected from the same site a decade earlier were inseminated ( $n = 109$ ; Yuval et al., 1996). For medfly females, 60–80% of females caught in traps in Hawaii were mated (McInnis et al., 1994). In yet another study conducted in the island of Chios, 73.7 and 63.4% ( $n = 1\ 014$ ) of captured females laid eggs (Kouloussis et al., 2011), but egg fertility was not reported.

At least for the medfly, the incidence of mating failure could reflect variation in the level of choosiness among individual females. For example, over five consecutive days 21% of laboratory-reared medfly females did not mate, although they were courted 1–67 times by 1–10 males. Among females that mated, 26% copulated with the first male that courted them. Approximately, 50% of females were courted by five or more males before mating, and only 7% of all courtships resulted in successful mounting and copulation (Whittier et al., 1994).

For lepidopteran species used in SIT, most data on female mating failure in the field are few and far between. Because their mating systems differ greatly from those of the better studied dipteran pests we are reluctant to generalize the patterns of female receptivity and mating failure across orders. Nevertheless, the few ecologically relevant studies are illuminating. In the almond moth, *Cadra cautella* (Walter), a pest of stored products, lifetime mating failure rate of females was 26%, a rate significantly influenced by the interaction between population size and sex ratio (McNamara et al., 2008). In the grapevine moth, *Lobesia botrana* (Denis & Schiffermüller), all females attracted to bait producing host odors had mated at least once, and fewer than 5% of females tested in the laboratory failed to copulate (Torres-Villa & Jennions, 2005). In another laboratory study of the same species, female mating failure ranged from 22–78%. This rate was significantly influenced by both male and female larval host, as individuals that emerged from Riesling grape cultivars had lower mating success than individuals from other cultivars (Moreau et al., 2007).

*Behavioral differences between sterile and wild males.* The SIT relies on wild female acceptance of mass-reared sterile males. However, discriminating wild females often reject sterile males at higher rates than wild males. For example, in a field cage study with medflies, only 8% of interactions resulted in wild females copulating with irradiated males vs. 28% of interactions with wild males (Lance et al., 2000). Indeed, field releases of male-only strains of sterile medflies have corroborated that wild females discriminate against sterile males (e.g., Shelly & Whittier, 1996). Female receptivity and willingness to mate will depend on adequate chemosensory signals from males, such as

pheromones, auditory or visual signals, and the intensity and amount of courtship. However, to date there is no consensus for what courtship components may determine a positive female response to a male (Lux et al., 2002a).

Selection pressures during colonization and mass-rearing processes often yield laboratory males that do not have the same courtship repertoire as their wild counterparts. Specifically, in medflies, mass-reared males perform courtship at different rates or in different sequences compared to wild males (Liimatainen et al., 1997; Cayol, 2000; Lance et al., 2000; Briceño & Eberhard, 2002). Shelly (2012) found that wild females discriminated against mass-reared males from a temperature-sensitive strain (*tsl*) and that this discrimination occurred before mounting, presumably because of shortened courtship that preceded mounting (see also Briceño & Eberhard, 2000). Quantitative differences in courtship behavior seem to result in wild females rejecting mass-reared males. Thus, mass-reared medfly males from Costa Rica were more likely to mount females without previous courtship than were wild males, and wild males initiated continuous vibration at significantly greater distances than did mass-reared males (Briceño & Eberhard, 2002). Mass-reared males from Costa Rica, Argentina, Hawaii (USA), and Mexico were slightly more likely to interrupt wing-buzzing (one element of the courtship sequence) than were wild flies, and this discontinuous courtship apparently led to fewer mountings (Briceño & Eberhard, 2002). In other studies, no specific differences have been found in behavioral repertoires between mass-reared and wild strains from different locations, yet up to 87% of wild females reject mass-reared males (Lux et al., 2002a).

Crowding conditions in mass-rearing can also alter the length and pattern of courtship, which can influence female mating decisions (Briceño & Eberhard, 1998). In medfly mass-reared and wild males, specific behavioral differences were observed in the duration of courtship, wing vibrations, and head rocks, which led to successful mountings (Briceño & Eberhard, 1998). For wild males, courtships that resulted in copulation were longer than courtships that were unsuccessful, whereas for mass-reared flies there were no significant differences between the lengths of successful or unsuccessful courtships (Briceño & Eberhard, 2000). However, in both these laboratory studies, male courtship was compared to females from the same strain. Thus, it would be interesting to compare courtship from mass-reared and wild males toward wild females.

In another study with Costa Rican medfly populations, wild flies were more aggressive than mass-reared flies, presumably because the intense crowding of mass-rearing selects for lower aggression (Briceño et al., 1999).

However, male-male aggression does not seem to be a factor determining mating success in medflies (Whittier et al., 1994; Shelly, 2000). For the Mexican fruit fly, *Anastrepha ludens* (Loew), crowding in early adulthood results in lower mating success. Wild males that experienced low male densities of 50 or 100 males per cage during 16 days post-emergence had more copulations than males from high-density cages of 500 males (Díaz-Fleischer et al., 2009). The decrease in sexual competitiveness due to crowding could be due to higher male-male interactions or to pheromone depletion, either of which could increase the probability of females rejecting males (Díaz-Fleischer et al., 2009).

In addition to the changes in sexual behavior that mass-rearing conditions can cause, irradiation can also affect courtship. For example, irradiated medfly males approached mass-reared females more than non-irradiated males did, provoking an aggressive response from the mass-reared female instead of engaging in normal courtship behavior (Lux et al., 2002b). This led to a higher frequency of rejection by the female. In comparison, non-irradiated males frequently remained still and allowed females to approach them and interact. Likewise, acoustic signals used during courtship can be affected by irradiation. For example, in the Queensland fruit fly, *Bactrocera tryoni* (Froggatt), the timing of acoustic signals, such as the pulse train intervals and durations of calling and courtship, differ between irradiated and non-irradiated males (Mankin et al., 2008). In the Caribbean fruit fly, *Anastrepha suspensa* (Loew), irradiation changes the waveform and frequency of the pre-copulatory courtship song performed by males (Sharp & Webb, 1977). However, we do not know whether this disrupts communication between sterile males and wild females.

Irradiation can also affect pheromone production and composition. For example, sterile Mexican fruit fly males produce less pheromone than non-irradiated males (Moreno et al., 1991), whereas sterile medfly males have reduced pheromone-calling activity compared to fertile males (Kraaijeveld & Chapman, 2004). However, even though the chemical composition of the pheromone can differ (Heath et al., 1994), there is also evidence that sterile medfly males call as frequently as wild males (Shelly et al., 1994; Shelly & Whittier, 1996). Shelly (1999) found that sterile medfly males from a 40-year-old, mass-reared strain called more often than fertile males from a young strain and so attracted more females. Moreover, the rate of female arrivals per minute of pheromone calling did not differ between the two strains, indicating no pronounced difference in the attractiveness of the chemical signal per se.

Female rejection of irradiated males has been observed in *A. gambiae* (Davidson et al., 1970), the melon fly,

*Bactrocera cucurbitae* (Coquillett) (Hibino & Iwahashi, 1991), and medfly (Shelly et al., 1994; McInnis et al., 1996, 2002; Shelly & Whittier, 1996). However, few studies have specifically tested the effect of irradiation by comparing mass-reared non-irradiated (fertile) vs. mass-reared irradiated (sterile) males mating with wild females. Of the only three medfly studies that have specifically tested irradiation effects, none have found an effect on medfly mating success (Wong et al., 1983; Barry et al., 2003; Shelly et al., 2005). Nevertheless, the last two studies used semi-wild flies from a sixth-generation stock. As mating competitiveness, and presumably female choosiness, can decrease with increasing laboratory generations (Iwahashi et al., 1983), additional studies on male irradiation using F1 wild individuals are needed. For Mexican fruit flies tested in a field cage, females were more likely to reject irradiated than non-irradiated or wild males (Moreno et al., 1991). In another study with this same species in a laboratory walk-in cage, wild females were more likely to mate with wild males than with mass-reared males irradiated at either 3 or 8 krad. However, there was no significant difference in the mating success of mass-reared, non-irradiated males compared to mass-reared, irradiated or wild males (Rull et al., 2005). Thus, differences in mating success cannot directly be attributed to irradiation or mass-rearing. In a field study of the codling moth, *Cydia pomonella* (L.), wild females were less likely to mate with mass-reared males, regardless of whether they had been irradiated or not, compared to wild males (Judd et al., 2006).

Female discrimination against sterile mass-reared males could also reflect population-wide evolution of increased female selectivity. In the context of a lengthy SIT program, the appearance of a heritable 'resistant type' in a female may lead to increased representation in the population as this genotype would confer a large fitness advantage over less-discriminating females (Boake et al., 1996; Robinson et al., 2009). McInnis et al. (1996) describe such a scenario for the medfly in Hawaii. Over a 4–5 year period of sterile male releases, wild females from Kauai Island showed a decreased tendency to select sterile males as mates. Moreover, wild females from Kauai showed a significantly higher rejection rate of sterile males than wild flies from other islands, indicating the evolution of behavioral resistance was not an archipelago-wide phenomenon but occurred only on Kauai. In species where female choice plays a major role, it is likely that there would be an increased chance for sexual isolation to evolve than when the male is the major determinant of mating success. For example, this is less likely to occur in species with swarming mating systems, such as anopheline mosquitoes, compared to lekking species where female choice is more prevalent (Robinson et al., 2009). Nevertheless, recent

studies on mate choice based on auditory dueting in mosquitoes suggest that the role of the females should not be underestimated (e.g., Cator et al., 2009).

*Spatial and temporal distribution.* The likelihood that wild females encounter sterile males is partially dependent upon the dispersal ability of released, sterile males and especially their ability to locate and join natural mating encounter sites, such as leks and swarms. In field cage trials, Cayol et al. (1999) and Lux et al. (2002b) both reported that sterile medfly males were resting and calling at sites different from those used by wild males, but the applicability of these observations to the wild is uncertain. In a field study in Hawaii (Shelly et al., 1994), released sterile medfly males successfully located and joined leks. However, this study involved ground releases over a small area, and comparable data from aerial releases over large areas are needed. Similarly, at large spatial scales (i.e., the whole orchard), sterile males matched the distribution pattern of wild males (Gavriel et al., 2012). At the tree level, where leks are formed, Whittier et al. (1992) observed that matings took place on many different plants, indicating that there was little variation in the ‘quality’ of different lek sites. Indeed, the quality of a medfly lek site within a tree changes throughout the day (Kaspi & Yuval, 1999). In contrast, in the Caribbean fruit fly, certain lek sites seem to be preferred, as matings were observed on leaves most proximal to the trunk (Sivinski, 1989). Modeling studies using this same species have highlighted factors, such as female choice, the quality of the sterile male, and mating competitiveness, in improving the encounter probability between sterile males and wild females in a lekking system (Hornig & Plant, 1992). The model suggests that even if territory defense ability is low, the probability of encounters may still be high if the proportion of sterile males participating in leks is high (Hornig & Plant, 1992).

Potential differences at a larger scale in the encounter rates of wild and sterile males may also influence the frequency with which wild females mate with the two male types. In the melon fly, mean dispersal ability ranged from 50 to 90 m, with a considerable overlap between wild and released males caught in traps. However, irradiation doses of 10 krad diminished dispersal distances (Hamada, 1980). In the codling moth, dispersal of irradiated males is shorter than that of wild males (Vreysen et al., 2010), and mobility decreases with increasing irradiation dose (Bloem et al., 2006), thus limiting male location of females. In contrast, in the Caribbean fruit fly, there was no significant difference in the flight duration and flight velocity of irradiated or non-irradiated males (Sharp & Webb, 1977). Likewise, in the sweet potato weevils *Cylas formicarius* Fabricius and

*Euscepes postfasciatus* (Fairmaire), there was no effect of irradiation on dispersal ability (Kumano et al., 2007, 2009a). Although flightless and obviously less vagile, weevil males nonetheless still need to orient toward potential mating partners in the field (Kumano et al., 2009a).

Several studies of the medfly have shown that, although a small fraction of released males may travel long distances, the great majority remains relatively close to the release point. Plant & Cunningham (1991) found that males remained within 150 m of the release point and that less than half the released populations survived beyond the third day. Meats & Smallridge (2007) found that 90% of released sterile males were trapped within 400–700 m of the release point, although a small percentage traveled up to 1 km. A similar pattern has been noted for the Queensland fruit fly, although in this case the distances traveled are slightly greater (Meats & Edgerton, 2008; Weldon & Meats, 2010). Sterile insect technique programs have recognized this pattern of dispersal and have adapted by using roving ground releases (Cunningham et al., 1980) or releases from aircraft (Rendon et al., 2004). As noted above, when released near natural leks, sterile males locate and join these aggregations (Shelly et al., 1994; Gavriel et al., 2012). Thus, assuming releases cover most of the targeted area, the dispersal ability of sterile fruit fly males should not be an important determinant of their encounter frequency with wild females.

At a temporal scale, reduced encounters might also result if sterile males show activity patterns different from those of wild flies. In several tephritid species, mass-reared males start their sexual activity earlier in the day relative to wild males (Papadopoulos et al., 1998; Weldon, 2005; Orozco-Dávila et al., 2007; Pérez-Staples et al., 2009). In the melon fly, albeit under laboratory conditions, mass-reared males have extended calling periods compared with wild males (Suzuki & Koyama, 1980). In medflies, wild males from Guatemala interacted with females earlier in the day compared with sterile males, whereas wild males from Kauai (Hawaii) interacted later in the day compared with sterile males (Lance et al., 2000). However, there was no difference in the time of participation between wild flies from Maui and Kona and sterile flies (Lance et al., 2000). Overall though, wild females rejected sterile males more readily than wild males (Lance et al., 2000). Similarly, sterile codling moth males initiate flight and mate finding approximately 45 min earlier than wild moths, which results in lower mating success (Judd et al., 2006). In the Queensland fruit fly, sterile males started mating much earlier in the evening than wild males, yet despite this there was no evidence of assortative mating between wild and sterile flies (Weldon, 2005; Pérez-Staples et al., 2009).

**Age.** Female choosiness may vary with age, which may affect the likelihood that wild females accept or reject a sterile male. For example, wild medfly female sexual activity peaks from 7 to 11 days of age and then decreases at 13 days of age (Liedo et al., 2002). Likewise, male age may affect the likelihood that a female accepts or rejects copulation. Thus, wild Mexican fruit fly females prefer to mate with older sexually experienced (non-virgin) males (Pérez-Staples et al., 2010). Working with semi-wild (non-irradiated) laboratory strains, two independent studies (Anjos-Duarte et al., 2011a; Shelly et al., 2011) have shown that young (<10 days old) medfly females prefer young males as mates, whereas old females (>20 days old) did not discriminate among males of various ages. This result suggests that, if some factor (e.g., low availability of dietary protein) delays female mating, older females may be less selective and thus more likely to mate with (non-preferred) sterile males. Experiments are needed to test this hypothesis. Furthermore, working with semi-wild flies, Shelly et al. (2012) have shown that young medfly males preferred younger (10 days old) over older (40 days old) virgin females. Thus again, if female mating is delayed for some reason, wild males may reject older, non-mated females, and this may serve to increase the likelihood that old females copulate with sterile males.

**Diet.** In insects, diet influences the growth of ovaries or egg production (ovogenesis) and thus initial primary mating receptivity. As tephritid flies are synovigenic (needing protein to mature), it is highly probable that not all females will find protein sources in nature (which are likely to be highly ephemeral) and will not mature sexually. Thus, high rates of mating failure may be evident in females that have long periods of pre-reproductive maturation (Rhainds, 2010). For example, wild Mexican and West Indies fruit fly, *Anastrepha obliqua* Macquart, females fed a continuous diet of yeast hydrolysate copulated 2 days earlier, on average, than females fed only sucrose every 3rd day (Aluja et al., 2008). Furthermore, as females have a long physiological maturation period that lasts from 1 week (medfly) to several weeks (*Bactrocera* spp. and *Anastrepha* spp.), they have a high risk of predation and death during this pre-reproduction period.

Post-teneral diet is also important for male reproductive success (Drew & Yuval, 2000). Evidence is accumulating from many tephritid species that protein-rich diets improve male ability to copulate with females and to inhibit their receptivity to additional copulations (Pereira et al., 2012; Taylor et al., in press). In mosquitoes, males require frequent sugar meals to fuel swarming (Yuval, 2006). Dietary adult supplements such as methylparaben can increase male longevity, but information on their

effect on mating performance is lacking (Benedict et al., 2009).

**Size.** Females may fail to mate with males if they are small [e.g., medflies: Churchill-Stanland et al. (1986), Taylor & Yuval (1999); Caribbean fruit flies: Burk (1983); West Indian sweet potato weevil: Kumano et al. (2011); but not Mexican fruit flies: Aluja et al. (2008)]. Field cage studies in medflies have shown that there is a clear preference for larger males irrespective of female size (Anjos-Duarte et al., 2011b). Thus, emphasis for mass-reared males should be made on producing large males. Whereas a standard diet during immature stages and controlled environmental conditions may yield mass-produced males with less size variation than wild males, factors such as larval crowding conditions (e.g., screwworm fly, *Cochliomyia hominivorax* Coquerel; Pitti et al., 2011) and irradiation [e.g., *Bactrocera zonata* (Saunders), Mahmoud & Barta, 2011], may decrease mass-reared adult or pupal size. Female preferences for sterile or wild males may fluctuate throughout the seasons, as sterile males may face greater competition in the field when environmental conditions produce large wild males (e.g., during cold months and or when high-quality food or hosts are available during immature stages; Navarro-Campos et al., 2011) than when smaller wild males are present. Additional field data on the effect of male size on female preferences are needed for other species controlled through SIT.

**Sex ratio.** In the context of SIT, an increase in overflooding ratios of sterile to wild males may promote the likelihood that females copulate with sterile males. The effectiveness of inducing sterility may also depend on whether the strain is bisexual or unisexual (male only) (e.g., McInnis et al., 1999; McInnis et al., 2004). For example, male-only releases of sterile medflies (VIENNA 4/Tol-94 strains) were more successful in inducing female sterility at sterile:wild release ratios of 100:1 compared to a bisexual strain (Rendon et al., 2004). McInnis et al. (1994) also found that male-only releases significantly caused higher sterility in wild medfly females than did bisexual medfly populations in Hawaii. In contrast, in another study conducted on medfly in Hawaii, Shelly & Whittier (1996) found that the proportion of matings achieved by sterile males did not differ between male-only and bisexual releases. The overflooding ratio will ultimately depend on the population growth rate, yet models exploring the proportion of sterile males needed to eradicate populations with lek mating systems emphasize the importance of quality over quantity of sterile males (Hornig & Plant, 1992). Field data on the wild population

sex ratio may be biased if it is estimated by the number of females caught in traps, as they do not take into account females that are not sexually mature and may not be attracted to baits.

#### Post-copulatory mating failure

Once mating has occurred, females that copulated with sterile males will be rendered infertile. Thus, ironically for SIT to be successful, female mating failure (in terms of reproduction) is realized in SIT only when females copulate with sterile males. Reproductive failure will be realized only if an adequate ejaculate (containing sufficient sperm and accessory gland products) is transferred from sterile males to wild females.

*Polyandry in wild females.* Multiple mating by wild females may decrease the efficiency of SIT. If females mate with a sterile male, which fails to inhibit female remating, the female can potentially seek and copulate with a wild male. Thus, reproductive failure will not be achieved. It is now clear that even for genera that have traditionally been thought of as monandrous, such as *Anastrepha* spp., that there is considerable variation in mating frequency by wild females (Aluja et al., 2009; Abraham et al., 2011a). For example, under laboratory conditions, the majority of wild Mexican fruit fly females mated only once in their lifetime, yet close to 10% mated more than four times (Aluja et al., 2009). Likewise, in the West Indian fruit fly females, approximately 20% of females mated twice, and a very small fraction mated three times (Aluja et al., 2009). Medfly females are also facultatively polyandrous (Mossinson & Yuval, 2003; Gavriel et al., 2009). Polyandry in wild females has been assessed in the Tobacco fly using microsatellite loci, which revealed that 22% of females produced progeny from two males (Song et al., 2007). Polyandry is also common in tsetse flies, where females can mate with up to three males (Bonomi et al., 2011) and in various species of Lepidopteran pests (Torres-Vila et al., 2004; Knight, 2007).

There is considerable inter-population variation in remating frequency in the medfly. Thus, in a Chios population, paternity analyses using microsatellite loci have reported remating frequencies of 3.8–21% (Bonizzoni et al., 2002) and 4–28% (Kraaijeveld et al., 2005), whereas Israeli populations had remating frequencies of up to 50% (Bonizzoni et al., 2006).

*Inhibition of female remating.* In tephritids, the ability of a male to inhibit female remating depends mainly on sperm and accessory gland products (Miyatake et al., 1999; Mossinson & Yuval, 2003; Radhakrishnan & Taylor, 2007; Abraham et al., 2012). Whereas sperm may reduce

receptivity in the short term, accessory gland products probably reduce receptivity in the longer term (Mossinson & Yuval, 2003; Gavriel et al., 2009). Irradiation affects spermatogenesis in all tephritid species studied, yet its effects on accessory gland products differ in published reports. Thus, although irradiation did not affect the production of accessory gland products in the Queensland fruit fly (e.g., Radhakrishnan et al., 2009), it had a significant effect on the ability of accessory gland homogenates from sterile male South American fruit flies, *Anastrepha fraterculus* (Wiedemann), to inhibit female receptivity (Abraham et al., 2012). A similar effect may be inferred from the reduced ability of sterile medflies to inhibit female receptivity (Kraaijeveld & Chapman, 2004; Gavriel et al., 2009; Morelli et al., in press). Furthermore, the sexual refractory period for medfly females is shorter when mated to sterile males than to wild males (Vera et al., 2003; Gavriel et al., 2009). Thus, the ability of a sterile male to induce female reproductive failure can be jeopardized.

Sterile male age is also an important factor that can determine their ability to induce reproductive failure. In medflies, sterile males of intermediate age (11 days old) inhibited female remating significantly more than younger or older flies (Gavriel et al., 2009). Another study also found that younger sterile males are less likely to inhibit female remating (Shelly et al., 2007).

*Sperm transfer.* Failure of sterile males to transfer adequate numbers of sperm could increase the probability that a wild female remates. If the female mates first with a sterile male and then remates with a wild male, reproductive failure will not be achieved. For example, medfly females that remated had less sperm than those that remained monandrous (Mossinson & Yuval, 2003). In certain species, full spermathecae may be a sufficient signal to remain monandrous. Mounting, however, does not assure copulation, and copulation does not guarantee insemination and sperm transfer (Briceño & Eberhard, 1998). In tsetse flies, sperm transfer or retention failure has been reported after normal copulation and spermatophore transfer (Briceño & Eberhard, 2009). Likewise, in laboratory-reared medflies and mass-reared Queensland fruit flies, 6 and 5% of copulations resulted in no sperm transfer (Taylor & Yuval, 1999; Pérez-Staples et al., 2007). Seo et al. (1990) found up to 38% of mass-reared medfly females had no sperm stored after mating, whereas for wild medflies only 1.5% of copulations resulted in no sperm storage (Taylor et al., 2000). For wild South American fruit flies, 32% of females mated with sugar-fed males and 12.5% mated with brewer's yeast-fed males had no spermatozoa in any of their three

spermathecae. In contrast, 7% of laboratory-reared females had no sperm after mating, and male diet had no effect on the proportion of females with empty spermathecae (Abraham et al., 2011b). In wild Caribbean fruit flies, only two mated females of 38 had no sperm stored in the spermathecae, but all had sperm in the ventral receptacle (Fritz, 2004). In *A. gambiae* up to 4% of wild females had no spermatozoa (Davidson et al., 1970). Although we cannot rule out that females may be ‘dumping’ sperm and not storing it, low or null sperm numbers potentially affect female’s propensity to remate. However, in West Indies and Queensland fruit flies, male accessory gland products seem to be more important than sperm in inducing sexual inhibition (Pérez-Staples et al., 2008; Radhakrishnan et al., 2009).

The capacity of sterile males to prevent female remating will be diminished if they transfer less sperm than wild males. Irradiation not only damages the chromosomes of the gonial cells (Bakri et al., 2005), but can also alter the quantity of sperm transferred to females. For example, non-irradiated medfly males transfer less sperm than irradiated males (Seo et al., 1990). In Melon and Queensland fruit flies, irradiated males become sperm depleted faster than non-irradiated males (Kuba & Ito, 1993; Radhakrishnan et al., 2009), whereas in the Mediterranean flour moth, *Ephesia kuehniella* Zeller, increasing radiation doses affected the number of both eupyrene and apyrene sperm (Koudelova & Cook, 2001). In contrast, in *E. postfasciatus* irradiation did not affect sperm production or viability, as irradiated males produced new sperm every day up to 16 days after irradiation (Kumano et al., 2008, 2009b). In the South American fruit fly, irradiation had no effect on the proportion of females with empty spermathecae (Allinghi et al., 2007), and in a recent medfly study there was no effect of irradiation on sperm transfer (M’saad Guerfali et al., 2011). The importance of the quantity of sperm transferred by irradiated males is dependent on how much it influences female remating behavior. Thus, more species-specific studies are needed to understand how sperm dynamics of irradiated males can determine wild female mating decisions.

*Identification of sperm from wild or sterile males.* A further complication in determining whether or not wild females are mating with sterile males is assessing whether sperm in their spermathecae derives from sterile or wild males. Initially, a technique was developed through which irradiated medfly sperm could be distinguished from wild fly sperm, as wild sperm heads are shorter, on average, than irradiated sperm heads (26 vs. 30  $\mu\text{m}$ , respectively; McInnis, 1993; McInnis et al., 1994). More recently, identification of sperm between wild and sterile males has

been made possible in medflies, Mexican fruit flies, and Caribbean fruit flies, through the production of transgenic males with fluorescent sperm (Scolari et al., 2008; Meza et al., 2011; Nirmala et al., 2011). These methods may allow a direct comparison of female mating rates with sterile or wild males, as fluorescent sperm can be observed inside female’s spermathecae using DsRed epifluorescence (Meza et al., 2011). Furthermore, fluorescent sperm can be detected in liquid traps for at least 2 weeks (Nirmala et al., 2011). Discrimination of sperm inside the female’s spermathecae is also possible between the Vienna-8 strain and wild type medflies using PCR markers (Andrés et al., 2007). For lepidopteran species, a cytological technique with orcein and Gamma stains allows the F1 progeny of irradiated and fertile males to be distinguished (Carpenter et al., 2009). Specimens are still distinguishable after being in sticky traps for 24 h (Wee et al., 2011).

#### Inducing mating failure by manipulating male quality

Several approaches have been developed and implemented in the last decade to improve the sexual performance of sterile males and thus induce female mating failure more effectively. Briefly, the approaches to manipulating males include altering the olfactory environment experienced by the males following eclosion, providing high-quality post-teneral nutrition (Yuval et al., 2007), inoculating males with probiotic bacteria, and (in species that experience a lengthy pre-reproductive period), inducing early maturation by applying hormones to young adults (review by Pereira et al., 2012). Advances in this field are currently restricted to a number of fruit fly species, but may be brought to bear on blood-sucking flies and lepidopterans as well.

#### Conclusion

A key component of SIT involves ‘building a better male’. Unfortunately, clear identification of the objective in no way facilitates its achievement. Despite considerable research, particularly on the Mediterranean fruit fly, there is still no consensus on which traits, individually or collectively, promote male mating success, and there is even less understanding of what traits wild females prefer. Indeed, for most species where SIT is used, we lack precise data on what percentage of the female population is mating with sterile males. Clearly, however, and as outlined above, the crowded conditions under which sterile fruit fly males are reared have selected for more rapid (shorter) courtship, which is often unacceptable to wild females. The prevailing approach emphasizes production volume to achieve higher overflooding ratios (sterile:wild males) in the field, despite the recognition of negative effects on male

courtship, it is unlikely that current rearing practices will change. Nonetheless, working within these constraints, studies on dietary or chemical manipulation of the adult environment can improve sterile male competitiveness. Such manipulations, based on detailed research, may be extended to additional pest and vector species, so that female mating failure can be considered a success.

## Acknowledgements

We thank Dinesh Rao and three anonymous referees for comments on the manuscript, and Ernesto Castañeda Barbosa and Nicolás Núñez Beverido for technical assistance with the literature.

## References

- Abraham S, Goane L, Rull J, Cladera J, Willink E & Vera MT (2011a) Multiple mating in *Anastrepha fraterculus* females and its relationship with fecundity and fertility. *Entomologia Experimentalis et Applicata* 141: 15–24.
- Abraham S, Goane LA, Cladera J & Vera MT (2011b) Effects of male nutrition on sperm storage and remating behavior in wild and laboratory *Anastrepha fraterculus* (Diptera: Tephritidae) females. *Journal of Insect Physiology* 57: 1501–1509.
- Abraham S, Cladera J, Goane L & Vera MT (2012) Factors affecting *Anastrepha fraterculus* female receptivity modulation by accessory gland products. *Journal of Insect Physiology* 58: 1–6.
- Allinghi A, Gramajo C, Willink E & Vilardi J (2007) Induction of sterility in *Anastrepha fraterculus* (Diptera: Tephritidae) by gamma radiation. *Florida Entomologist* 90: 96–102.
- Aluja M, Pérez-Staples D, Sivinski J, Sánchez A & Piñero J (2008) Effects of male condition on fitness in two tropical tephritid flies with contrasting life histories. *Animal Behaviour* 76: 1997–2009.
- Aluja M, Rull J, Sivinski J, Trujillo G & Pérez-Staples D (2009) Male and female condition influence mating performance and sexual receptivity in two tropical fruit flies (Diptera: Tephritidae) with contrasting life histories. *Journal of Insect Physiology* 55: 1091–1098.
- Andrés VS, Urbaneja A, Sabater-Muñoz B & Castañera P (2007) A novel molecular approach to assess mating success of sterile *Ceratitidis capitata* (Diptera: Tephritidae) males in sterile insect technique programs. *Journal of Economic Entomology* 100: 1444–1449.
- Anjos-Duarte C, Costa A & Joachim-Bravo I (2011a) Influence of female age on variation of mate choice behavior in Mediterranean fruit fly (Diptera: Tephritidae). *Journal of Insect Behavior* 24: 11–21.
- Anjos-Duarte CS, Costa AM & Joachim-Bravo IS (2011b) Sexual behaviour of the Mediterranean fruit fly (Diptera: Tephritidae): the influence of female size on mate choice. *Journal of Applied Entomology* 135: 367–373.
- Bakri A, Mehta K & Lance D (2005) Sterilizing insects with ionizing radiation. *Sterile Insect Technique, Principles and Practice in Area-Wide Integrated Pest Management* (ed. by VA Dyck, J Hendrichs & AS Robinson), pp. 233–268. Springer, Dordrecht, The Netherlands.
- Barry JD, McInnis DO, Gates D & Morse JG (2003) Effects of irradiation on Mediterranean fruit flies (Diptera: Tephritidae): emergence, survivorship, lure attraction, and mating competition. *Journal of Economic Entomology* 96: 615–622.
- Benedict MQ, Knols BGJ, Bossin HC, Howell PI, Mialhe E et al. (2009) Colonisation and mass rearing: learning from others. *Malaria Journal* 8(Suppl 2): S4.
- Bloem S, Carpenter JE & Dorn S (2006) Mobility of mass-reared diapaused and nondiapaused *Cydia pomonella* (Lepidoptera: Tortricidae): effect of mating status and treatment with gamma radiation. *Journal of Economic Entomology* 99: 699–706.
- Boake CRB, Shelly TE & Kaneshiro KY (1996) Sexual selection in relation to pest-management strategies. *Annual Review of Entomology* 41: 211–229.
- Bonizzoni M, Katsoyannos BI, Marguerie R, Guglielmino CR, Gasperi G et al. (2002) Microsatellite analysis reveals remating by wild Mediterranean fruit fly females, *Ceratitidis capitata*. *Molecular Ecology* 11: 1915–1921.
- Bonizzoni M, Gomulski LM, Mossinson S, Guglielmino CR, Malacrida AR et al. (2006) Is polyandry a common event among wild populations of the pest *Ceratitidis capitata*? *Journal of Economic Entomology* 99: 1420–1429.
- Bonomi A, Bassetti F, Gabrieli P, Beadell J, Falchetto M et al. (2011) Polyandry is a common event in wild populations of the tsetse fly *Glossina fuscipes fuscipes* and may impact population reduction measures. *PLoS Neglected Tropical Diseases* 5: e1190.
- Briceño RD & Eberhard WG (1998) Medfly courtship duration: a sexually selected reaction norm changed by crowding. *Ethology, Ecology & Evolution* 10: 369–382.
- Briceño RD & Eberhard WG (2000) Possible fisherian changes in female mate-choice criteria in a mass-reared strain of *Ceratitidis capitata* (Diptera: Tephritidae). *Annals of the Entomological Society of America* 93: 343–345.
- Briceño RD & Eberhard WG (2002) Decisions during courtship by male and female medflies (Diptera: Tephritidae): correlated changes in male and female acceptance criteria in mass-reared flies. *Florida Entomologist* 85: 14–31.
- Briceño RD & Eberhard WG (2009) Experimental demonstration of possible cryptic female choice on male tsetse fly genitalia. *Journal of Insect Physiology* 55: 989–996.
- Briceño RD, Ramos D & Eberhard WG (1999) Aggressive behavior in medflies (*Ceratitidis capitata*) and its modification by mass rearing (Diptera: Tephritidae). *Journal of the Kansas Entomological Society* 72: 17–27.
- Burk T (1983) Behavioral ecology of mating in the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae). *Florida Entomologist* 66: 330–344.
- Cardé RT & Minks AK (1995) Control of moth pests by mating disruption: successes and constraints. *Annual Review of Entomology* 40: 559–585.
- Carpenter JE, Marti OG, Wee SL & Suckling DM (2009) Cytological attributes of sperm bundles unique to F(1) progeny of irra-

- diated male Lepidoptera: relevance to sterile insect technique programs. *Florida Entomologist* 92: 80–86.
- Cator LJ, Arthur BJ, Harrington LC & Hoy RR (2009) Harmonic convergence in the love songs of the dengue vector mosquito. *Science* 323: 1077–1079.
- Cayol JP (2000) Changes in sexual behavior and life history traits of tephritid species caused by mass-rearing processes. *Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior* (ed. by M Aluja & AL Norrbom), pp. 843–860. CRC Press, Boca Raton, FL, USA.
- Cayol JP, Vilardi J, Rial E & Vera MT (1999) New indices and method to measure the sexual compatibility and mating performance of *Ceratitidis capitata* (Diptera: Tephritidae) laboratory-reared strains under field cage conditions. *Journal of Economic Entomology* 92: 140–145.
- Charlwood JD, Pinto J, Sousa C, Ferreira C, Petrarca V & Rosario VD (2003) 'A mate or a meal'—pre-gravid behaviour of female *Anopheles gambiae* from the islands of Sao Tome and Principe, West Africa. *Malaria Journal* 2: 9.
- Churchill-Stanland C, Stanland R, Wong TTY, Tanaka N, McInnis DO & Dowell RV (1986) Size as a factor in the mating propensity of Mediterranean fruit flies, *Ceratitidis capitata* (Diptera: Tephritidae) in the laboratory. *Journal of Economic Entomology* 79: 614–619.
- Cunningham RT, Routhier W, Harris EJ, Cunningham G, Johnson L et al. (1980) Eradication of medfly by sterile male release. *Citrograph* 65: 63–69.
- Davidson G, Odetoyinbo JA, Colussa B & Coz J (1970) A field attempt to assess the mating competitiveness of sterile males produced by crossing 2 member species of the *Anopheles gambiae* complex. *Bulletin World Health Organization* 42: 55–67.
- Díaz-Fleischer F, Arredondo J & Aluja M (2009) Enriching early adult environment affects the copulation behaviour of a tephritid fly. *Journal of Experimental Biology* 212: 2120–2127.
- Drew RAI & Yuval B (2000) The evolution of fruit fly feeding behavior. *Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior* (ed. by M Aluja & A Norrbom), pp. 731–749. CRC Press, Boca Raton, FL, USA.
- Fritz AH (2004) Sperm storage patterns in singly mated females of the Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae). *Annals of the Entomological Society of America* 97: 1328–1335.
- Gavriel S, Gazit Y & Yuval B (2009) Remating by female Mediterranean fruit flies (*Ceratitidis capitata*, Diptera: Tephritidae): temporal patterns and modulation by male condition. *Journal of Insect Physiology* 55: 637–642.
- Gavriel S, Gazit Y, Leach A, Mumford J & Yuval B (2012) Spatial patterns of sterile medfly dispersal. *Entomologia Experimentalis et Applicata* 142: 17–26.
- Hamada R (1980) Studies on the dispersal behavior of melon flies, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae), and the influence of gamma-irradiation on dispersal. *Applied Entomology and Zoology* 15: 363–371.
- Heath RR, Epsky ND, Dueben B, Guzman A & Andrade LE (1994) Gamma radiation effect on production of four pheromonal components of male Mediterranean fruit flies (Diptera: Tephritidae). *Journal of Economic Entomology* 87: 904–909.
- Hendrichs J, Kenmore P, Robinson AS & Vreysen MJB (2007) Area-wide integrated pest management (AW-IPM): principles, practice and prospects. *Area-Wide Control of Insect Pests: From Research to Field Implementation* (ed. by MJB Vreysen, AS Robinson & J Hendrichs), pp. 3–34. Springer, Vienna, Austria.
- Hibino Y & Iwahashi O (1991) Appearance of wild females unresponsive to sterilized males on Okinawa Is. in the eradication program of the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). *Applied Entomology and Zoology* 26: 265–270.
- Hornig S-B & Plant R (1992) Impact of lek mating on the sterile insect technique: a modeling study. *Researches on Population Ecology* 34: 57–76.
- Iwahashi O, Ito Y & Shiyomi M (1983) A field evaluation of the sexual competitiveness of sterile melon flies, *Dacus (Zeugodacus) cucurbitae*. *Ecological Entomology* 8: 43–48.
- Judd GJR, Thistlewood HMA, Gardiner MGT & Lannard BL (2006) Is lack of mating competitiveness in spring linked to mating asynchrony between wild and mass-reared codling moths from an operational sterile insect programme? *Entomologia Experimentalis et Applicata* 120: 113–124.
- Kaspi R & Yuval B (1999) Mediterranean fruit fly leks: factors affecting male location. *Functional Ecology* 13: 539–545.
- Knight AL (2007) Multiple mating of male and female codling moth (Lepidoptera: Tortricidae) in apple orchards treated with sex pheromone. *Environmental Entomology* 36: 157–164.
- Knipling EF (1955) Possibilities of insect control or eradication through the use of sexual sterile males. *Journal of Economic Entomology* 48: 459–462.
- Koudelova J & Cook PA (2001) Effect of gamma radiation and sex-linked recessive lethal mutations on sperm transfer in *Ephestia kuehniella* (Lepidoptera: Pyralidae). *Florida Entomologist* 84: 172–182.
- Kouloussis NA, Papadopoulos NT, Katsoyannos BI, Müller H-G, Wang J-L et al. (2011) Seasonal trends in *Ceratitidis capitata* reproductive potential derived from live-caught females in Greece. *Entomologia Experimentalis et Applicata* 140: 181–188.
- Kraaijeveld K & Chapman T (2004) Effects of male sterility on female remating in the Mediterranean fruit fly, *Ceratitidis capitata*. *Proceedings of the Royal Society of London B* 271: S209–S211.
- Kraaijeveld K, Katsoyannos BI, Stavrinides M, Kouloussis NA & Chapman T (2005) Remating in wild females of the Mediterranean fruit fly, *Ceratitidis capitata*. *Animal Behaviour* 69: 771–776.
- Kuba H & Ito Y (1993) Remating inhibition in the melon fly, *Bactrocera* (= *Dacus*) *cucurbitae* (Diptera: Tephritidae): copulation with spermless males inhibits female remating. *Journal of Ethology* 11: 23–28.
- Kumano N, Kohama T & Ohno S (2007) Effect of irradiation on dispersal ability of male sweetpotato weevils (Coleoptera: Brentidae) in the field. *Journal of Economic Entomology* 100: 730–736.

- Kumano N, Haraguchi D & Kohama T (2008) Effect of irradiation on mating performance and mating ability in the West Indian sweetpotato weevil, *Euscepes postfasciatus*. *Entomologia Experimentalis et Applicata* 127: 229–236.
- Kumano N, Kawamura F, Haraguchi D & Kohama T (2009a) Irradiation does not affect field dispersal ability in the West Indian sweetpotato weevil, *Euscepes postfasciatus*. *Entomologia Experimentalis et Applicata* 130: 63–72.
- Kumano N, Haraguchi D & Kohama T (2009b) Sperm storage and viability within females of *Euscepes postfasciatus*: effect of irradiation on sperm abundance and viability within female. *Journal of Insect Physiology* 55: 813–817.
- Kumano N, Kuriwada T, Shiramoto K, Haraguchi D & Kohama T (2011) Effect of body size and sex ratio on male alternative mating tactics of the West Indian sweetpotato weevil, *Euscepes postfasciatus*. *Entomologia Experimentalis et Applicata* 135: 154–161.
- Lance DR & McInnis DO (2005) Biological basis of the sterile insect technique. *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management* (ed. by VA Dyck, J Hendrichs & AS Robinson), pp. 69–94. Springer, Dordrecht, The Netherlands.
- Lance DR, McInnis DO, Rendon P & Jackson CG (2000) Courtship among sterile and wild *Ceratitis capitata* (Diptera: Tephritidae) in field cages in Hawaii and Guatemala. *Annals of the Entomological Society of America* 93: 1179–1185.
- Liedo P, De Leon E, Barrios MI, Valle-Mora JF & Ibarra G (2002) Effect of age on the mating propensity of the Mediterranean fruit fly (Diptera: Tephritidae). *Florida Entomologist* 85: 94–101.
- Liimatainen J, Hoikkala A & Shelly T (1997) Courtship behavior in *Ceratitis capitata* (Diptera: Tephritidae): comparison of wild and mass-reared males. *Annals of the Entomological Society of America* 90: 836–843.
- Lux SA, Munyiri FN, Vilardi JC, Liedo P, Economopoulos A et al. (2002a) Consistency in courtship pattern among populations of medfly (Diptera: Tephritidae): comparisons among wild strains and strains mass reared for SIT operations. *Florida Entomologist* 85: 113–125.
- Lux SA, Vilardi JC, Liedo P, Gaggi K, Calcagno GE et al. (2002b) Effects of irradiation on the courtship behaviour of medfly (Diptera, Tephritidae) mass reared for the sterile insect technique. *Florida Entomologist* 85: 102–112.
- Mahmoud MF & Barta M (2011) Effect of gamma radiation on the male sterility and other quality parameters of peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). *Horticultural Science* 38: 54–62.
- Mankin RW, Lemon M, Harmer AMT, Evans CS & Taylor PW (2008) Time-pattern and frequency analyses of sounds produced by irradiated and untreated male *Bactrocera tryoni* (Diptera: Tephritidae) during mating behavior. *Annals of the Entomological Society of America* 101: 664–674.
- McInnis DO (1993) Size differences between normal and irradiated sperm heads in mated female Mediterranean fruit flies (Diptera: Tephritidae). *Annals of the Entomological Society of America* 86: 305–308.
- McInnis DO, Tam S, Grace C & Miyashita D (1994) Population suppression and sterility rates induced by variable sex ratio, sterile insect releases of *Ceratitis capitata* (Diptera: Tephritidae). *Annals of the Entomological Society of America* 87: 231–240.
- McInnis DO, Lance DR & Jackson CG (1996) Behavioral resistance to the sterile insect technique by Mediterranean fruit fly (Diptera: Tephritidae) in Hawaii. *Annals of the Entomological Society of America* 89: 739–744.
- McInnis DO, Rendon P, Jang E, van Sauer-Muller A, Sugayama R & Malavasi A (1999) Interspecific mating of introduced, sterile *Bactrocera dorsalis* with wild *B. carambolae* (Diptera: Tephritidae) in Suriname: a potential case for cross-species sterile insect technique. *Annals of the Entomological Society of America* 92: 758–765.
- McInnis DO, Rendon P & Komatsu J (2002) Mating and remating of medflies (Diptera: Tephritidae) in Guatemala: individual fly marking in field cages. *Florida Entomologist* 85: 126–137.
- McInnis DO, Tam S, Lim R, Komatsu J, Kurashima R & Albrecht C (2004) Development of a pupal color-based genetic sexing strain of the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). *Annals of the Entomological Society of America* 97: 1026–1033.
- McNamara KB, Elgar MA & Jones TM (2008) Causes and consequences of variation in female mating frequency in the almond moth, *Cadra cautella*. *Behaviour* 145: 779–793.
- Meats A & Edgerton JE (2008) Short- and long-range dispersal of the Queensland fruit fly, *Bactrocera tryoni* and its relevance to invasive potential, sterile insect technique and surveillance trapping. *Australian Journal of Experimental Agriculture* 48: 1237–1245.
- Meats A & Smallridge CJ (2007) Short- and long-range dispersal of medfly, *Ceratitis capitata* (Dipt., Tephritidae), and its invasive potential. *Journal of Applied Entomology* 131: 518–523.
- Meza JS, Nirmala X, Zimowska GJ, Zepeda-Cisneros CS & Handler AM (2011) Development of transgenic strains for the biological control of the Mexican fruit fly, *Anastrepha ludens*. *Genetica* 139: 53–62.
- Miyatake T, Chapman T & Partridge L (1999) Mating induced inhibition of remating in female Mediterranean fruit flies. *Journal of Insect Physiology* 45: 1021–1028.
- Moreau J, Thiery D, Troussard JP & Benrey B (2007) Grape variety affects female but also male reproductive success in wild European grapevine moths. *Ecological Entomology* 32: 747–753.
- Morelli R, Paranhos BJ, Coelho AM, Castro R, Garziera L et al. (in press) Exposure of sterile Mediterranean fruit fly (Diptera: Tephritidae) males to ginger root oil reduces female remating. *Journal of Applied Entomology*. doi: 10.1111/j.1439-0418.2010.01584.x
- Moreno DS, Sanchez M, Robacker DC & Worley J (1991) Mating competitiveness of irradiated Mexican fruit-fly (Diptera, Tephritidae). *Journal of Economic Entomology* 84: 1227–1234.
- Mossinson S & Yuval B (2003) Regulation of sexual receptivity of female Mediterranean fruit flies: old hypotheses revisited and a new synthesis proposed. *Journal of Insect Physiology* 49: 561–567.

- M'saad Guerfali M, Parker A, Fadhl S, Hemdane H, Raies A & Chevrier C (2011) Fitness and reproductive potential of irradiated mass-reared Mediterranean fruit fly males *Ceratitidis capitata* (Diptera: Tephritidae): lowering radiation doses. *Florida Entomologist* 94: 1042–1050.
- Navarro-Campos C, Martínez-Ferrer MT, Campos JM, Fibla JM, Alcaide J et al. (2011) The influence of host fruit and temperature on the body size of adult *Ceratitidis capitata* (Diptera: Tephritidae) under laboratory and field conditions. *Environmental Entomology* 40: 931–938.
- Nirmala X, Olson SR, Holler TC, Cho KH & Handler AM (2011) A DsRed fluorescent protein marker under polyubiquitin promoter regulation allows visual and amplified gene detection of transgenic Caribbean fruit flies in field traps. *BioControl* 56: 333–340.
- Orozco-Dávila D, Hernández R, Meza S & Domínguez J (2007) Sexual competitiveness and compatibility between mass-reared sterile flies and wild populations of *Anastrepha ludens* (Diptera: Tephritidae) from different regions in Mexico. *Florida Entomologist* 90: 19–26.
- Papadopoulos NT, Katsoyannos BI, Kouloussis NA, Economopoulos AP & Carey JR (1998) Effect of adult age, food, and time of day on sexual calling incidence of wild and mass-reared *Ceratitidis capitata* males. *Entomologia Experimentalis et Applicata* 89: 175–782.
- Pereira R, Yuval B, Liedo P, Teal PEA, Shelly TE et al. (2012) Improving sterile male performance in support of programmes integrating the sterile insect technique against fruit flies. *Journal of Applied Entomology*. doi: 10.1111/j.1439-0418.2011.01664.x
- Pérez-Staples D, Harmer AMT & Taylor PW (2007) Sperm storage and utilization in female Queensland fruit flies (*Bactrocera tryoni*). *Physiological Entomology* 32: 127–135.
- Pérez-Staples D, Aluja M, Macias-Ordonez R & Sivinski J (2008) Reproductive trade-offs from mating with a successful male: the case of the tephritid fly *Anastrepha obliqua*. *Behavioral Ecology and Sociobiology* 62: 1333–1340.
- Pérez-Staples D, Weldon CW, Smallridge CJ & Taylor P (2009) Pre-release feeding on yeast hydrolysate enhances sexual competitiveness of sterile male Queensland fruit flies in field cages. *Entomologia Experimentalis et Applicata* 131: 159–166.
- Pérez-Staples D, Martínez-Hernández MG & Aluja M (2010) Male age and experience increases mating success but not female fitness in the Mexican fruit fly. *Ethology* 116: 778–786.
- Pitti A, Skoda SR, Kneeland KM, Berkebile DR, Molina-Ochoa J et al. (2011) Effect of adult screwworm male size on mating competence. *Southwestern Entomologist* 36: 47–60.
- Plant RE & Cunningham RT (1991) Analyses of the dispersal of sterile Mediterranean fruit flies (Diptera: Tephritidae) released from a point source. *Environmental Entomology* 20: 1493–1503.
- Radhakrishnan P & Taylor PW (2007) Seminal fluids mediate sexual inhibition and short copula duration in mated female Queensland fruit flies. *Journal of Insect Physiology* 53: 741–745.
- Radhakrishnan P, Pérez-Staples D, Weldon CW & Taylor PW (2009) Multiple mating and sperm depletion in male Queensland fruit flies: effects on female remating behaviour. *Animal Behaviour* 78: 839–846.
- Rendon P, McInnis D, Lance D & Stewart J (2004) Medfly (Diptera: Tephritidae) genetic sexing: large-scale field comparison of males-only and bisexual sterile fly releases in Guatemala. *Journal of Economic Entomology* 97: 1547–1553.
- Rhainds M (2010) Female mating failures in insects. *Entomologia Experimentalis et Applicata* 136: 211–226.
- Robinson AS, Knols BGJ, Voigt G & Hendrichs J (2009) Development of the sterile insect technique for African malaria vectors: conceptual framework and rationale. *Malaria Journal* 8(Suppl 2): S1.
- Rull J, Brunel O & Méndez ME (2005) Mass rearing history negatively affects mating success of male *Anastrepha ludens* (Diptera: Tephritidae) reared for sterile insect technique programs. *Journal of Economic Entomology* 95: 1510–1516.
- Scolari F, Schetelig MF, Bertin S, Malacrida AR, Gasperi G & Wimmer EA (2008) Fluorescent sperm marking to improve the fight against the pest insect *Ceratitidis capitata* (Wiedemann, Diptera: Tephritidae). *New Biotechnology* 25: 76–84.
- Seo ST, Vargas RI, Gilmore JE, Kurashima RS & Fujimoto MS (1990) Sperm transfer in normal and gamma-irradiated, laboratory-reared Mediterranean fruit flies (Diptera: Tephritidae). *Journal of Economic Entomology* 83: 1949–1953.
- Sharp JL & Webb JC (1977) Flight performance and signalling sound of irradiated or unirradiated *Anastrepha suspensa*. *Proceedings of the Hawaiian Entomological Society* 22: 525–532.
- Shelly TE (1999) Trimedlure and the mating competitiveness of irradiated male Mediterranean Fruit Flies (Diptera: Tephritidae). *Environmental Entomology* 28: 780–786.
- Shelly TE (2000) Aggression between wild and laboratory-reared sterile males of the Mediterranean fruit fly in a natural habitat. *Florida Entomologist* 83: 105–108.
- Shelly TE (2012) Mate choice by wild and mass-reared females of the Mediterranean fruit fly. *Journal of Applied Entomology* 136: 238–240.
- Shelly TE & Whittier TS (1996) Mating competitiveness of sterile male Mediterranean fruit flies (Diptera: Tephritidae) in male-only releases. *Annals of the Entomological Society of America* 89: 754–758.
- Shelly TE, Whittier TS & Kaneshiro KY (1994) Sterile insect release and the natural mating system of the Mediterranean fruit fly, *Ceratitidis capitata* (Diptera: Tephritidae). *Annals of the Entomological Society of America* 87: 470–481.
- Shelly TE, Edu J & Pahio E (2005) Lack of an irradiation effect on the mating performance of mass-reared males of the Mediterranean fruit fly. *Florida Entomologist* 88: 547–548.
- Shelly TE, Edu J & Pahio E (2007) Age-dependent variation in mating success of sterile male Mediterranean fruit flies (Diptera: Tephritidae): implications for sterile insect technique. *Journal of Economic Entomology* 100: 1180–1187.
- Shelly T, Edu J & Pahio E (2011) Female medflies mate selectively with young males but gain no apparent fitness benefits. *Journal of Insect Behavior* 24: 55–66.

- Shelly T, Edu J & Pahio E (2012) Mate choice by lekking males: evidence from the Mediterranean fruit fly. *Annals of the Entomological Society of America* 105: 368–376.
- Sivinski J (1989) Lekking and the small-scale distribution of the sexes in the Caribbean fruit fly, *Anastrepha suspensa* (Loew). *Journal of Insect Behavior* 2: 3–13.
- Song SD, Drew RAI & Hughes JM (2007) Multiple paternity in a natural population of a wild tobacco fly, *Bactrocera cacuminata* (Diptera: Tephritidae), assessed by microsatellite DNA markers. *Molecular Ecology* 16: 2353–2361.
- Suzuki Y & Koyama J (1980) Temporal aspects of mating behavior of the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae): a comparison between laboratory and wild strains. *Applied Entomology and Zoology* 15: 215–224.
- Taylor PW & Yuval B (1999) Postcopulatory sexual selection in Mediterranean fruit flies: advantages for large and protein-fed males. *Animal Behaviour* 58: 247–254.
- Taylor PW, Kaspi R & Yuval B (2000) Copula duration and sperm storage in Mediterranean fruit flies from a wild population. *Physiological Entomology* 25: 94–99.
- Taylor PW, Pérez-Staples D, Weldon CW, Collins SR, Fanson BG et al. (in press) Post-teneral nutrition as an influence on reproductive development, sexual performance and longevity of Queensland fruit flies. *Journal of Applied Entomology*. doi: 10.1111/j.1439-0418.2011.01644.x
- Torres-Vila L, Rodríguez-Molina MC & Jennions M (2004) Polyandry and fecundity in the Lepidoptera: can methodological and conceptual approaches bias outcomes? *Behavioral Ecology and Sociobiology* 55: 315–324.
- Torres-Villa L & Jennions M (2005) Male mating history and female fecundity in the Lepidoptera: do male virgins make better partners? *Behavioral Ecology and Sociobiology* 57: 318–326.
- Tripet F, Toure YT, Dolo G & Lanzaro GC (2003) Frequency of multiple inseminations in field-collected *Anopheles gambiae* females revealed by DNA analysis of transferred sperm. *American Journal of Tropical Medicine and Hygiene* 68: 1–5.
- Tripet F, Lounibos LP, Robbins D, Moran J, Nishimura N & Blosser EM (2011) Competitive reduction by satyriization? Evidence for interspecific mating in nature and asymmetric reproductive competition between invasive mosquito vectors. *American Journal of Tropical Medicine and Hygiene* 85: 265–270.
- Vera MT, Cladera JL, Calcagno G, Vilardi JC & McInnis DO (2003) Remating of wild *Ceratitidis capitata* (Diptera: Tephritidae) females in field cages. *Annals of the Entomological Society of America* 96: 563–570.
- Voordouw MJ, Koella JC & Hurd H (2008) Comparison of male reproductive success in malaria-refractory and susceptible strains of *Anopheles gambiae*. *Malaria Journal* 7: 103.
- Vreysen MJB, Hendrichs J & Enkerlin W (2006) The sterile insect technique as a component of sustainable area-wide integrated pest management of selected horticultural insect pests. *Journal of Fruit and Ornamental Plant Research* 14: 107–131.
- Vreysen MJB, Carpenter JE & Marec F (2010) Improvement of the sterile insect technique for codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera Tortricidae) to facilitate expansion of field application. *Journal of Applied Entomology* 134: 165–181.
- Wall R & Langley PA (1993) The mating behaviour of tsetse flies (Glossina): a review. *Physiological Entomology* 18: 211–218.
- Wee S-L, Suckling DM & Barrington AM (2011) Feasibility study on cytological sperm bundle assessment of F1 progeny of irradiated male painted apple moth (*Teia anartoides* Walker; Lepidoptera: Lymantriidae) for the sterile insect technique. *Australian Journal of Entomology* 50: 269–275.
- Weldon CW (2005) Mass-rearing and sterilisation alter mating behaviour of male Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *Australian Journal of Entomology* 44: 158–163.
- Weldon C & Meats A (2010) Dispersal of mass-reared sterile, laboratory-domesticated and wild male Queensland fruit flies. *Journal of Applied Entomology* 134: 16–25.
- Whittier TS, Kaneshiro KY & Prescott LD (1992) Mating behavior of Mediterranean fruit flies (Diptera: Tephritidae) in a natural environment. *Annals of the Entomological Society of America* 85: 214–218.
- Whittier TS, Nam FY, Shelly TE & Kaneshiro KY (1994) Male courtship success and female discrimination in the Mediterranean fruit fly (Diptera: Tephritidae). *Journal of Insect Behavior* 7: 159–170.
- Wong TTY, Nishimoto JI & Couey HM (1983) Mediterranean fruit fly (Diptera: Tephritidae): further studies on selective mating response of wild and of unirradiated and irradiated, laboratory-reared flies in field cages. *Annals of the Entomological Society of America* 76: 51–55.
- Yuval B (2006) Mating systems of blood-feeding flies. *Annual Review of Entomology* 51: 413–440.
- Yuval B, Blay S & Kaspi R (1996) Sperm transfer and storage in the Mediterranean fruit fly (Diptera: Tephritidae). *Annals of the Entomological Society of America* 89: 486–492.
- Yuval B, Maor M, Levy K, Kaspi R, Taylor P & Shelly T (2007) Breakfast of champions or kiss of death? Survival and sexual performance of protein-fed, sterile Mediterranean fruit flies (Diptera: Tephritidae). *Florida Entomologist* 90: 115–122.