Optimizing methyl-eugenol aromatherapy to maximize posttreatment effects to enhance mating competitiveness of male *Bactrocera carambolae* (Diptera: Tephritidae)

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**Abstract** Methyl-eugenol (ME) (1,2-dimethoxy-4-(2-propenyl)benzene), a natural phytochemical, did enhance male *Bactrocera carambolae* Drew & Hancock (Diptera: Tephritidae) mating competitiveness 3 d after ingestion. Enhanced male mating competitiveness can significantly increase the effectiveness of the sterile insect technique (SIT). ME application to mass reared sterile flies by feeding is infeasible. ME application by aromatherapy however, would be a very practical way of ME application in fly emergence and release facilities. This approach was shown to enhance mating competitiveness of *B. carambolae* 3 d posttreatment (DPT). Despite this added benefit, every additional day of delaying release will reduce sterile fly quality and will add cost to SIT application. The present study was planned to assess the effects of ME-aromatherapy on male *B. carambolae* mating competitiveness 1DPT and 2DPT. ME aromatherapy 1DPT or 2DPT did enhance mating competitiveness of *B. carambolae* males whereas ME feeding 1DPT and 2DPT did not. Male mating competitiveness was enhanced by the ME aromatherapy irrespective if they received 1DPT, 2DPT or 3DPT. ME aromatherapy, being a viable approach for its application, did enhance mating competitiveness of male *B. carambolae* 1 d posttreatment as ME feeding did 3 d after ingestion.

**Key words** *Bactrocera carambolae*; mating competitiveness; ME aromatherapy; methyl-eugenol; ME feeding; posttreatment effect; SIT

**Introduction**

The carambola fruit fly *Bactrocera carambolae* Drew & Hancock (Diptera: Tephritidae) is native to Indonesia, Malaysia, and Thailand. It was detected in 1975 in Paramaribo, Suriname, South America, and has since spread to Guyana, French Guiana, and northeastern Brazil (Godoy, 2006; van Sauer-Müller, 2008). It is a pest of economic importance, causing severe economic losses to more than 150 fruit species and has been declared a quarantine pest insect in the Caribbean region, interfering with international trade of fruits and vegetables (Malavasi et al., 2000). *B. carambolae* males are attracted to methyl-eugenol (ME) (1,2-dimethoxy-4-(2-propenyl)benzene) (Iwahashi et al., 1996), which is a phenylpropanoid compound found in > 450 plant species (Metcalf & Metcalf, 1992; Tan & Nishida, 2012). ME is a powerful attractant for males of many tropical tephritid fruit fly species of the genera *Bactrocera* and *Dacus* (Drew, 1974, 1989; White & Elson-Harris, 1992; Shelly, 2010) and is routinely used, not only to monitor, but also to control populations of *B. carambolae* with an environmentally friendly lure and kill approach termed “male annihilation technique” (MAT)
(Steiner et al., 1970; van Sauers-Müller, 2008). MAT based on ME is being established to curtail establishment of *B. carambolae* in northeastern Brazil (Malavasi et al., 2000; Godoy, 2006).

MAT has been used to suppress many *Bactrocera* pest populations as part of integrated pest management programmes and even to eradicate isolated populations, such as on islands or during outbreaks, for example, *Bactrocera dorsalis* Hendel from the Marianas Islands, Micronesia (Steiner et al., 1970), *B. dorsalis* from the Okinawa Islands, Japan (Koyama et al., 1984), and various *Bactrocera* spp. incursions in California (USA) and elsewhere (Chambers, 1977; CDFA, 2013). However, MAT failed to eradicate *B. dorsalis* from the Ogasawara Islands of Japan (Christenson, 1963), perhaps owing to the evolution of nonresponse to ME among wild males (Iwahashi, 1973). The efficacy of the MAT may also be compromised where wild males have access to abundant natural ME sources as consumption of sufficient amounts of the chemical can result in reduced attraction to ME baited traps (Shelly, 1997). Population suppression or eradication relying only on MAT may be more difficult when applied against species that are less responsive to ME.

Based on laboratory measurements on males of several sibling species of the *B. dorsalis* complex, *B. carambolae* males show comparatively low sensitivity to ME (Wee et al., 2002). Similarly, field data revealed that 4–5 times as many ME fiber blocks per hectare were needed to suppress populations of *B. carambolae* compared to the number typically used to reduce populations of *B. dorsalis* (van Sauers-Müller, 2008). Thus, integration of the SIT after suppression by MAT application is most likely a more effective strategy to eradicate certain *Bactrocera* populations as was demonstrated on the Mariana Islands (Steiner et al., 1970). Furthermore, MAT integration with the SIT is also an option to avoid long-term pressure on a population by selecting for less ME-responsive males (Cunningham, 1989).

The SIT, another environmentally friendly technique, involves mass-rearing of male insects, sterilizing them by ionizing radiation, and releasing them on a sustained and area-wide basis over the target area in numbers large enough to outcompete their wild counterparts (Knipling, 1955; Dyck et al., 2005). Wild females that mate with sterile males produce no off-spring, and therefore the release of sterile males in adequate numbers reduces the wild population. In certain cases, this population suppression can lead to eventual eradication of the target population (Hendrichs & Robinson, 2009). Because the success of the SIT depends on the ability of mass-reared, sterile males to obtain copulations with wild females, it is essential that the mass-reared sterile males be competitive with wild males. Unfortunately, long-term mass-rearing reduces the overall mating competitiveness of male fruit flies (Cayol, 2000), necessitating the release of very large numbers of males which adds substantially to the cost of SIT. Reduced male mating performance in many tephritids can be overcome by improving colony management (Robinson & Hendrichs, 2005) and the adult diet (Pereira et al., 2013; Taylor et al., 2013) as well as by exposing sterile males to certain chemicals, such as ME for many of the *Bactrocera* spp. (Shelly, 2010; Shelly et al., 2010 Pereira et al., 2013). Studies on various *Bactrocera* spp., including *B. carambolae* have shown that males fed ME produce more attractive pheromone and have higher mating success than control, unfed males (Wee & Tan, 2000). Thus, prerelease ME feeding may serve to increase the effectiveness of SIT against *Bactrocera* pest species. During field enclosure tests, Shelly et al. (2010) demonstrated that the same level of egg sterility could be induced with 84% fewer ME treated sterile *B. dorsalis* males as compared to ME deprived males. Furthermore, ME exposed males were less likely than nonexposed wild males to be recaptured in ME-baited traps (Shelly, 1994). Low incidence of repeated feeding by ME exposed sterile males would allow the application of MAT and SIT application simultaneously. Thus, a “male replacement” approach (reducing the wild males at ME traps while simultaneously releasing sterile males) would significantly increase the sterile to wild male overflooding ratios and reduce the number of sterile males that need to be released (Robinson & Hendrichs, 2005; McInnis et al., 2011; Barclay et al., 2014).

The main limitation for the use of ME at fly emergence and release facilities is the lack of a suitable delivery system to mass-reared sterile adult male flies before their release in the field. The currently used methods for emerging and holding fruit flies prior to field release (Mabry, 1986; Salvato et al., 2004; Enkerlin, 2007) do not support the inclusion of any ME feeding to adult flies. Exposure to ME has to be brief in view of ME’s potential toxicity (Steiner, 1952). Tan & Tan (2013) designed a machine to allow only a short feeding time to sterile males using a ME-impregnated relaying belt where males are brushed off and collected. While this was an innovative system for testing under experimental conditions, it is not suitable for treating millions of sterile males per day on an industrial scale. In search for a practical way of applying ME to male *B. carambolae* in holding and emergence facilities, Haq et al. (2014) demonstrated that exposure to the aroma of ME (a procedure termed ME aromatherapy) enhanced male *B. carambolae* mating competitiveness 3 d after treatment, similar to ME feeding. This posttreatment interval was selected, because previous work on ME feeding showed...
that mating enhancement in B. carambolae males was not apparent until at least 3 d after treatment (in contrast to B. dorsalis, where males enjoy elevated mating ability as early as 1 d postfeeding). The demonstration of effective aromatherapy opened new possibilities for the practical use of ME, but the apparent need to hold B. carambolae males for 3 d postexposure has potentially detrimental consequences, that is, additional holding time may reduce the vigor of the sterile males and will certainly increase programmatic costs owing to prolonged maintenance requirements. The objective of this study therefore was to analyze whether ME aromatherapy can enhance male B. carambolae mating success on day 1 or 2 after treatment.

**Material and methods**

**Study insects**

The B. carambolae flies used in this study originated from Paramaribo, Suriname, and had been cultured for 27 generations at the FAO/IAEA Insect Pest Control Laboratory of the Agriculture and Biotechnology Laboratories in Seibersdorf, Austria. The colony was maintained on a carrot powder-based larval diet that was modified from the standard (wheat bran-based) Seibersdorf diet (Hooper, 1987). Following emergence, the adult flies were provided with standard diet (sugar : hydrolyzed yeast in a 3 : 1 proportion) and supplied with water *ad libitum*. The flies were sexed within 3 d after emergence (well before reaching sexual maturity at day 15 postemergence; Haq, unpublished data), transferred to a plexiglass tubular cage (20 cm × 64 cm diameter) having both openings covered with cloth mesh, and supplied with standard adult diet and water *ad libitum*. The flies were maintained in the laboratory at 24 ± 1 °C, 60% ± 5% RH and a photoperiod of 14 L : 10 D. All the flies used in a given experiment were from the same batch of pupae. Males were marked 1 d earlier to ME treatment by holding nonanaesthetized individuals motionless in nylon netting and applying water-based paint to the thorax.

**Treatments**

**ME-feeding** ME-feeding was conducted in a room isolated from the fly culture room. Marked males (15–21 d old, n = 100) were transferred to a plexiglass tubular cage (20 cm × 64 cm diameter) having both openings covered with cloth mesh. ME (0.5 mL) was placed on a filter paper strip, which was then placed in a Petri dish (15 cm diameter) and introduced in the cage. The males were allowed to feed on the ME (hereafter called ME-fed males) for 1 h (09:30–10:30h; peak ME foraging time; Wee & Tan, 2000). The Petri dish containing the ME was then removed, and the treated filter paper strip sealed in a polythene bag and discarded. The males were again provided with standard adult diet and water *ad libitum*.

**ME-aromatherapy** ME-aromatherapy was carried out in another room isolated from the fly culture room and the ME-feeding room. Marked males (15–21 d old, n = 100) were transferred to a plexiglass tubular cage (20 cm × 64 cm diameter) having both openings covered with cloth mesh. ME (0.5 mL) was introduced in the same manner as described above, except that the Petri dish was covered with fine nylon mesh that prevented male contact with the ME source. The males were exposed to ME volatiles (hereafter called ME-aroma-treated males) for 3 h (09:30–12:30h). The males started to move away from the Petri dish after 3 h and slight shaking of Petri dish resulted in all males fly away. Therefore, ME exposure for 3 h was adopted. The Petri dish was then removed, and the ME-laden paper strip sealed in a polythene bag and discarded. The males were again provided with standard adult diet and water *ad libitum*.

**No-ME treatment** Male flies that were not exposed to any ME treatment (hereafter called ME-deprived males) were maintained on standard adult diet and water *ad libitum* in another room isolated from the rooms used for ME-feeding or ME-aromatherapy. Untreated males were marked on the same day as the treated males and maintained in cages in the same manner as the treated males. Untreated males were of the same age as that of treated males. Female flies were not exposed to ME and maintained on standard adult diet and water *ad libitum* in the same room.

**Field cages**

The field cages used for mating trials were screened, circular tents (2.2 m high × 2 m diameter) (Calkins & Webb, 1983), each containing a potted citrus tree of 2 m height. Eight such field cages were placed inside a large insect greenhouse (24 m × 10 m × 4 m) that allowed us to carry out 8 replicates of the test simultaneously. A temperature of 26 ± 2 °C and 60% ± 5% RH was maintained throughout the experiment. Field cage tests took place within an insect greenhouse with a seminatural illumination due to a translucent roof.

**Experiments**

**Experiment 1. Mating competitiveness of ME-aroma-treated-males-2DPT and ME-fed-males-2DPT**

Twenty ME-aroma-treated-males-2DPT and 20 ME-fed-males-2DPT were released simultaneously in a field cage...
90 min before sunset. Males of the congeneric species *B. cucurbitae* and *B. dorsalis* start pheromone calling approximately 90 min before sunset (Arakaki et al., 1984), and similar time was therefore selected for the current trials. Fifteen minutes after releasing the male flies, 20 virgin females (same age as that of males) were introduced into the field cages. Thus, the male : female sex ratio in a given field cage was 2 : 1 (40 : 20). Two observers (1 observer assigned for 4 cages) collected the mating pairs from 8 field cages by visiting each cage after every 10 min. The pairs were collected and coaxed separately in each vial and left there to complete their mating. Eight replicates were evaluated simultaneously.

Experiment 2. Mating competitiveness of ME-aroma-treated-males-2DPT, ME-fed-males-2DPT and ME-deprived males The same protocol used in experiment 1 was followed, except that ME-aroma-treated-males-2DPT, ME-fed-males-2DPT and ME-deprived males were competing for mating with virgin females (i.e., in each cage, 60 males were competing for 20 females). Eight replicates were evaluated simultaneously.

Experiment 3. Mating competitiveness of ME-aroma-treated-males-1DPT and ME-deprived males The same protocol used in experiment 1 was followed, except that ME-aroma-treated-males-1DPT and ME-deprived males were competing for mating with virgin females and 5 replicates were evaluated simultaneously.

Experiment 4. Mating competitiveness of ME-aroma-treated-males-1DPT and ME-fed-males-1DPT The same protocol used in experiment 1 was followed, except that ME-aroma-treated-males-1DPT and ME-fed-males-1DPT, and 8 replicates were evaluated simultaneously.

Experiment 5. Mating competitiveness of ME-aroma-treated-males-1DPT, ME-fed-males-1DPT and ME-deprived males The same protocol used in experiment 1 was followed, except that ME-aroma-treated-males-1DPT, ME-fed-males-1DPT, and ME-deprived males were competing for mating with virgin females (i.e., in each cage, 60 males were competing for 20 females). Eight replicates were evaluated simultaneously.

Experiment 6. Mating competitiveness of ME-aroma-treated-males-2DPT, ME-aroma-treated-males-2DPT, and ME-aroma-treated-males-3DPT The same protocol used in experiment 1 was followed, except that ME-aroma-treated-males-1DPT, ME-aroma-treated-males-2DPT, and ME-aroma-treated-males-3DPT were competing for mating with virgin females (i.e., in each cage, 60 males were competing for 20 females). Eight replicates were evaluated simultaneously.

Experiment 7. Mating competitiveness of ME-aroma-treated-males-1DPT and ME-fed-males-3DPT Procedures were similar to the preceding experiments, except that ME-aroma-treated-males-1DPT were competing with ME-fed-males-3DPT for mating with virgin females (i.e., in each cage, 40 males were competing for 20 females). Thirteen replicates were evaluated in 2 d (8 and 5, respectively).

Data analyses Differences in relative mating success (number of matings out of total possible matings) between 2 treatments (subjected to parametric assumptions) were analyzed by the unpaired *t*-test. Differences in relative mating success among 3 treatments (subjected to parametric assumptions) were analyzed by one-way ANOVA. The significant value used in data analysis was 95% (α = 0.05). Complementary pairwise comparisons of means were performed by Tukey’s test (Ott & Longnecker, 2001).

Results

Experiment 1. Mating competitiveness of ME-aroma-treated-males-2DPT and ME-fed-males-2DPT

ME-aroma-treated-males-2DPT achieved significantly more matings (*t* = 2.37, df = 14, *P* < 0.05) than ME-fed-males-2DPT (Fig. 1).
Table 1  Relative mating success (% ± SE) of *Bactrocera carambolae* ME-aroma-treated-males-2DPT (days posttreatment), ME-fed-males-2DPT, and ME-deprived-males competing for mating with virgin females under field cages conditions. In another comparison ME-aroma-treated-males-1DPT, ME-fed-males-1DPT, and ME-deprived-males were competing for mating with virgin females. Eight replicates were performed for both experiments, and in each replicate 20 males of each treatment competed for 20 females. For each experiment, means followed by different letters differed significantly (Tukey’s test, $P < 0.05$).

| Mating% (± SE) | 2 d posttreatment (DPT) | 1 d posttreatment (DPT) |
|---------------|-------------------------|-------------------------|
| ME-aroma-treated-males | ME-fed-males | ME-deprived-males | ME-aroma-treated-males | ME-fed-males | ME-deprived-males |
| 40.6 (± 4.4) A | 25 (± 3.5) B | 33.1 (± 3.3) AB | 45.6 (± 3.3) A | 18.1 (± 1.6) C | 33.1 (± 2.9) B |

Fig. 2 Relative mating success (% of total matings per replicate) of ME-deprived and ME-aroma-treated *Bactrocera carambolae* males 1 d posttreatment (1DPT). In each replicate, 20 males of each treatment were competing for 20 virgin females under field cages conditions. Symbols represent raw data for 5 replicates; horizontal lines represent mean ± SE (unpaired t-test, df = 8, $P < 0.001$).

Experiment 2. Mating competitiveness of ME-aroma-treated-males-2DPT, ME-fed-males-2DPT and ME-deprived males

There were significant differences in mating success among the different male treatments ($F = 4.73$, df = 23, $P < 0.05$). ME-aroma-treated-males-2DPT achieved significantly more matings (Tukey’s Test, $P < 0.05$) than ME-fed-males-2DPT, but mating success by either of these treated males was not significantly different from that of ME-deprived males (Table 1).

Experiment 3. Mating competitiveness of ME-aroma-treated-males-1DPT and ME-deprived males

ME-aroma-treated-males-1DPT achieved significantly higher ($t = 6.26$, df = 8, $P < 0.001$) mating success than ME-deprived males (Fig. 2).

Fig. 3 Relative mating success (% of total matings per replicate) of ME-aroma-treated and ME-fed *Bactrocera carambolae* males 1 d posttreatment (1DPT). In each replicate, 20 males of each treatment were competing for 20 virgin females under field cages conditions. Symbols represent raw data for 8 replicates; horizontal lines represent mean ± SE (unpaired t-test, df = 14, $P < 0.001$).

Experiment 4. Mating competitiveness of ME-aroma-treated-males-1DPT and ME-fed-males-1DPT

ME-aroma-treated-males-1DPT achieved significantly higher ($t = 6.76$, df = 14, $P < 0.001$) mating success than ME-fed-males-1DPT (Fig. 3).

Experiment 5. Mating competitiveness of ME-aroma-treated-males-1DPT, ME-fed-males-1DPT, and ME-deprived males

There were significant differences in mating success among the differently treated males ($F = 25.17$, df = 23, $P < 0.001$). ME-aroma-treated-males-1DPT obtained the highest mating success, followed by ME-deprived males and finally ME-fed-males-1DPT (Tukey’s Test, $P < 0.05$) (Table 1).
Experiment 6. Mating competitiveness of ME-aroma-treated-males-1DPT, ME-aroma-treated-males-2DPT, and ME-aroma-treated-males-3DPT

There was no difference ($F = 1.28$, df = 23, $P = 0.29$) in mating success by ME-aroma-treated-males-1DPT or 2DPT or 3DPT (Fig. 4).

Experiment 7. Mating competitiveness of ME-aroma-treated-males-1DPT and ME-fed-males-3DPT

There was no difference ($t = 0.97$, df = 24, $P = 0.33$) in mating success by ME-aroma-treated-males-1DPT or ME-fed-males-3DPT (Fig. 5).

Discussion

Previous work by Haq et al. (2014) supported the findings of Wee et al. (2007) that, in tests performed 3 d after exposure, ME-feeding enhances the mating competitiveness of *B. carambolae* males relative to control, unfed males. Furthermore, Haq et al. (2014) demonstrated that exposure to ME volatiles (ME aromatherapy) also increased male mating success 3 d after treatment. The results presented in this paper show that ME aromatherapy also enhanced mating competitiveness of *B. carambolae* males 1 or 2 d after treatment, unlike ME feeding which did not enhance male mating success during those days. On the contrary, ME feeding reduced male mating success 1 d after feeding as compared to ME-deprived males. ME aromatherapy enhanced male mating success 1DPT as it did 2DPT and 3DPT. Mating success by ME-aroma-treated-males 1 d after exposure was as high as that of ME-fed-males 3 d after exposure.

The positive effect of ME aromatherapy on male mating success 1DPT or 2DPT was consistent, except for experiment 2 where ME-aroma-treated-males-2DPT, ME-fed-males-2DPT, and ME-deprived males were competing for mating with virgin females; in this case mating success by ME-aroma-treated-males-2DPT was significantly higher than ME-fed-males-2DPT but similar to that of ME-deprived males. This anomaly might have reflected the fact that the negative effect of ME feeding was greater 1DPT than 2DPT, such that the greater mating success of ME-fed-males-2DPT caused a greater reduction in the relative mating success by ME-aroma-treated-males-2DPT. This impact may have lessened the difference in mating success of ME-aroma-treated-males-2DPT and ME-deprived males, resulting in a nonsignificant difference. Our observation that ME feeding 1DPT or 2 DPT had no positive effect on mating is consistent with the findings by Wee et al. (2007), who also showed that ME effect on enhanced mating success of *B. carambolae* males was not visible 1DPT or 2DPT but was apparent at 3DPT. However, this was not the case for ME application by aromatherapy, where ME-aroma-treated males attained higher mating success equally for 1DPT, 2DPT, and 3DPT.

Understanding ME consumption, metabolism, sensitivity, and efficient utilization is required to understand differential postfeeding effects on mating success in different
Bactrocera species and the application of aromatherapy as a potential substitute to feeding in SIT programs. B. dorsalis males attained higher mating success 1 d after ME feeding, while B. carambolae males appear to attain a higher mating success over ME-deprived males 3 d after feeding (Wee et al., 2007). This discrepancy may be related to a differential ME metabolite(s) accumulation rate in rectal glands of the 2 species (Wee & Tan, 2007), that is, whereas in both species ME metabolites increase with time, there was a significant decrease in the accumulation rate (defined as accumulation in rectal glands after partial release of the metabolite(s) in pheromones during the courtship period) 3-d post-ME feeding for B. carambolae compared with 1 d in B. dorsalis males.

Despite the finding that ME feeding enhances male B. carambolae mating success, there is at present, no effective way of providing this chemical to large numbers of sterile males. Haq et al. (2014) demonstrated that exposure to ME aroma boosts male mating success provides a potential solution to prerelase chemical treatment analogous to ginger root oil aromatherapy that is now routinely used in many Mediterranean fruit fly emergence and release facilities in the world (USDA, 2009). As now shown in this paper, ME aromatherapy would have the supplementary advantage that these treated B. carambolae males can be released on the same or next day of the treatment. Enhanced mating success due to ME exposure will reduce the numbers of sterile males that need to be released, which will reduce the cost of SIT applications (McInnis et al., 2011). Furthermore, reducing the period that males are kept in the facility postaroma treatment will further increase the cost effectiveness of a SIT programme. If ME application by aromatherapy also reduces the incidence of B. carambolae sterile males from responding to ME sources and repeat feeding in the field (Shelly, 1994), this would allow applying simultaneously both MAT and SIT resulting in a synergetic control strategy that again could increase dramatically control efficiency and cost reduction (Barclay et al., 2014).

B. carambolae males respond to ME at the onset of sexual maturity, approximately 15 d after emergence. Holding males for such a long time in a holding facility would still be very impractical and costly. In the successful SIT programme in the Okinawa Prefecture, Japan, the congeneric B. cucurbitae (Coquillet) mass-reared sterile males, which have a similar sexual maturity age, were only kept for 3 d in the release facility and then released in the field (Nakamori & Kuba, 1990). In operational SIT programmes, the sterile flies should be kept in the fly emergence and release facility until close to sexual maturity (which is costly) but releasing them before they attain sexual maturity will reduce the efficiency of the programme as many males may die before being able to mate with wild females. Haq et al. (2010) demonstrated that treating B. cucurbitae genetic sexing strain males with juvenile hormone analogue and dietary protein can accelerate sexual maturation by approximately 8 d. As such, these males can be held in the facility for 4–5 d and then be released at the onset of their sexual maturity. Therefore, the potential of accelerating sexual maturation in B. carambolae males by juvenile hormone analogue treatment in combination with ME aromatherapy should be evaluated.

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Disclosure

The authors have no conflicts of interest.

References

Arakaki, N., Kuba, H. and Soemori, H. (1984) Mating behavior of the Oriental fruit fly, Dacus dorsalis Hendel (Diptera: Tephritidae). Applied Entomology and Zoology, 19, 42–51.

Barclay, H.J., McInnis, D.O. and Hendrichs, J. (2014) Modeling the area-wide integration of male annihilation and the simultaneous release of methyl-eugenol-exposed Bactrocera spp. sterile males. Annals of the Entomological Society of America, 107, 97–112.

Cayol, J.P. and Webb, J.C. (1983) A cage and support framework for behavioral tests of fruit flies in the field. Florida Entomologist, 66, 512–514.

Christenson, L.D. (1963) The male-annihilation technique in the control of exotic fruit flies. Advances in Chemistry, 41, 431–435.

©2014 The Authors Journal compilation © Institute of Zoology, Chinese Academy of Science, 22, 661–669
Cunningham, R.T. (1989) Male annihilation. Fruit Flies, Their Biology, Natural Enemies and Control, World Crop Pests 3B (eds. A.S. Robinson & G.H.S. Hooper), pp. 345–351. Elsevier, The Netherlands.

Drew, R.A.I. (1974) The responses of fruit fly species in the South Pacific area to male attractants. Journal of Australian Entomological Society, 13, 267–270.

Drew, R.A.I. (1989) The Tropical Fruit Flies (Diptera: Tephritidae: Dacinae) of the Australasian and Oceanian Regions, pp. 521. Queensland Museum, Australia.

Dyck, V.A., Hendrichs, J. and Robinson, A.S. (2005) The Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management. Springer, Dordrecht, The Netherlands.

Enkerlin, W. (2007) Guidance for Packing, Shipping, Holding and Release of Sterile Flies in Area-Wide Fruit Fly Control Programmes. FAO/IAEA, Rome, Italy.

Godoy, M.J.S. (2006) The carambola fruit fly program in state of Amapa, Brazil. 7th International Symposium on Fruit Flies of Economic Importance. Bahia, Brazil, ID 372–381.

Haq, I., Cacéres, C., Hendrichs, J, Teal, P.E.A., Wornoayporn, V., Stauffer, C. and Robinson, A.S. (2010) Effects of the juvenile hormone analogue methoprene and dietary protein on male melon fly Bactrocera cucurbitae (Diptera: Tephritidae) mating success. Journal of Insect Physiology, 56, 1503–1509.

Haq, I., Vreysen, M.J.B., Cacéres, C., Shelly, T.E. and Hendrichs, J. (2014) Methyl eugenol aromatherapy enhances the mating competitiveness of male Bactrocera carambolae Drew & Hancock (Diptera: Tephritidae). Journal of Insect Physiology, 68, 1–6.

Hendrichs, J. and Robinson, A.S. (2009) Sterile insect technique. Encyclopedia of Insects, 2nd edn (eds. V.H. Resh & R.T. Cardé), pp. 953–957. Academic Press, USA.

Hooper, G.H.S. (1987) Application of quality control procedures to large scale rearing of the Mediterranean fruit fly. Entomologia Experimentalis et Applicata, 44, 161–167.

Iwahashi, O. (1973) Ecological Studies on the Oriental Fruit Fly, Dacus dorsalis, in the Ogawara Islands. Tokyo Metropolitan Government Publication, Japan.

Iwahashi, O., Syamusdin-Subahar, T.S. and Sastrodihardjo, S. (1996) Attractiveness of methyl eugenol to the fruit fly Bactrocera carambolae (Diptera: Tephritidae) in Indonesia. Annals of the Entomological Society of America, 89, 653–660.

Knipling, E.F. (1955) Possibilities of insect control or eradication through the use of sexually sterile males. Journal of Economic Entomology, 48, 459–469.

Koyama, J., Teruya, T. and Tanaka, K. (1984) Eradication of oriental fruit fly (Diptera: Tephritidae) from the Okinawa islands by a male annihilation method. Journal of Economic Entomology, 77, 468–472.

Mabry, H.E. (1986) Sterile fruit fly emergence containers. Unpublished Memorandum, Assistant Director of Survey and Emergency Response Staff.

Malavasi, A., van Sauers-Müller, A., Midgarden, D., Kellman, V., Didelot, D., Caplon, P. and Ribeiro, O. (2000) Regional programme for the eradication of the carambola fruit fly in South America. Area-Wide Control of Fruit Flies and Other Insect Pests (ed. K.H. Tan), pp. 395–399. Panerbit Universiti, Sains Malaysia.

McInnis, D.O., Kurashima, R., Shelly, T.E., Komatsu, J, Edu, J. and Pahio, E. (2011) Pre-release exposure to methyl eugenol increases the mating competitiveness of sterile males of the Oriental fruit fly (Diptera: Tephritidae) in a Hawaiian orchard. Journal of Economic Entomology, 104, 1969–1978.

Metcalf, R.L. and Metcalf, E.R. (1992) Plant Kairomones in Insect Ecology and Control. Chapman and Hall, New York, USA.

Nakamori, H. and Kuba, H. (1990) Aerial distribution of sterile melon flies, Dacus cucurbitae Coquillett, anesthetized by chilling. Japan Agricultural Research Quarterly, 24, 31–36.

Ott, R.L. and Longneaker, M. (2001) An Introduction of Statistics Methods and Data Analyses, 5th edn., Duxbury Publishers, Pacific Grove, CA, USA.

Pereira, R., Yuval, B., Liedo, P, Teal, P.E.A., Shelly, T.E., McInnis, D.O. and Hendrichs, J. (2013) Improving sterile male performance in support of programmes integrating the sterile insect technique against fruit flies. Journal of Applied Entomology, 137s1, 178–190.

Robinson, A.S. and Hendrichs, J. (2005) Prospects for the future development and application of the sterile insect technique Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management (eds. V.A. Dyck, J. Hendrichs & A.S. Robinson), pp. 727–760. Dordrecht, The Netherlands.

Salvato, M., Holler, T., Worley, J. and Stewart, J. (2004) Efficacy of tower medfly eclosion systems. Biocontrol Science and Technology, 14, 77–80.

Shelly, T.E. (2010) Effects of methyl eugenol and raspberry ketone/cue lure on the sexual behavior of Bactrocera species (Diptera: Tephritidae). Applied Entomology and Zoology, 45, 349–361.

Shelly, T.E. (1994) Consumption of methyl eugenol by male Bactrocera dorsalis (Diptera: Tephritidae): low incidence of repeat feeding. Florida Entomologist, 77, 201–208.

Shelly, T.E. (1997) Selection for non-responsiveness to methyl eugenol in male Oriental fruit flies (Diptera: Tephritidae). Florida Entomologist, 80, 248–253.

Shelly, T.E., Edu, J. and McInnis, D.O. (2010) Pre-release consumption of methyl eugenol increases the mating competitiveness of sterile males of the Oriental fruit fly, Bactrocera dorsalis, in large field enclosures. Journal of Insect Science, 10, doi: 10.1673/031.010.0801.
Steiner, L.F. (1952) Methyl eugenol as an attractant for the Oriental fruit fly. *Journal of Economic Entomology*, 45, 241–248.

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

Tan, K.H. and Nishida, R. (2012) Methyl eugenol: Its occurrence, distribution, and role in nature, especially in relation to insect behavior and pollination. *Journal of Insect Science*, 12, doi: 10.1673/031.012.5601.

Tan, L.T. and Tan, K.H. (2013) Automated tephritid fruit fly semiochemical mass feeding structure: design, construction and testing. *Journal of Applied Entomology*, 137, 217–229.

Taylor, P.W., Pérez-Staples, D., Weldon, C.W., Collins, S.R., Fanson, B.G., Yap, S. and Smallridge, C. (2013) Post-teneral nutrition as an influence on reproductive development, sexual performance and longevity of Queensland fruit flies. *Journal of Applied Entomology*, 137s1, 113–125.

USDA (United States Department of Agriculture) (2009) Final Report United States, Mexico, and Guatemala Fruit Fly Emergence and Release Facilities Review. Riverdale, MD.

van Sauers-Müller, A. (2008) Carambola fruit fly situation in Latin America and the Caribbean. *Proceedings of the Caribbean Food Crops Society*, 44, 135–144.

Wee, S.L., Hee, A.K.W. and Tan, K.H. (2002) Comparative sensitivity to and consumption of methyl eugenol in three *Bactrocera dorsalis* (Diptera: Tephritidae) complex sibling species. *Chemoeconology*, 12, 193–197.

Wee, S.L. and Tan, K.H. (2000) Sexual maturity and intraspecific mating success of two sibling species of the *Bactrocera dorsalis* complex. *Entomologia Experimentalis et Applicata*, 94, 133–139.

Wee, S.L. and Tan, K.H. (2007) Temporal accumulation of phenylpropanoids in male fruit flies, *Bactrocera dorsalis* and *B. carambolae* (Diptera: Tephritidae) following methyl eugenol consumption. *Chemoeconology*, 17, 81–85.

Wee, S.L., Tan, K.H. and Nishida, R. (2007) Pharmacophagy of methyl eugenol by males enhances sexual selection of *Bactrocera carambolae*. *Journal of Chemical Ecology*, 33, 1272–1282.

White, I.M. and Elson-Harris, M.M. (1992) *Fruit Flies of Economic Significance: Their Identification and Bionomics*. CAB International, London, UK.