ESTABLISHMENT OF NON-METHYL EUGENOL-ATTRACTED MALES OF ORIENTAL FRUIT FLY, *Bactrocera dorsalis* (Hendel) SUPPLEMENTED WITH SEMIOCHEMICALS

By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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December 2021

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The Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is one of the world's most destructive and invasive pest of fruits. Control of this insect has relied heavily on the Male Annihilation Technique (MAT), and the Sterile Insect Technique (SIT). Combined use of MAT and SIT offers excellent control of *B. dorsalis*. However, issues with sterile males getting killed by going to those MAT traps have reduced the efficacy of both techniques. Nonetheless, the availability of non-ME-responding male *B. dorsalis* would enable the simultaneous application of MAT and SIT. The objectives of this study were to establish domesticated *B. dorsalis* lines and screen them for non-attraction to methyl eugenol (ME), to ascertain further those non-ME-attraction phenotypic expression, to evaluate the attraction of any non-ME-responding (NR) males to semiochemicals such as zingerone (ZN) and β–caryophyllene (BCP), and to evaluate the effects of consuming those semiochemicals by males on their mating success. Thus, as a part of a laboratory-scale proof of concept, the possibility of raising NR males of *B. dorsalis* that were supplemented with exposure to those compounds for enhanced mating advantage against wild males was explored. First, domesticated lines of *B. dorsalis* colonies reared from larvae in infested *Syzygium* sp. fruits were established and those males screened for non-ME responsiveness. Flies were collected from four different locations and those males were exposed to ME successively for a total of 9 days in the morning with 24 h interval in between each day of exposure. Non-attraction of males to ME was defined as absence of attraction in at least two successive exposures. Two separate lines of NR males from those locations were established. Second, the phenotypic expression of non-ME-responsiveness was ascertained by assessing lure response and mating competitiveness of NR males. Two NR lines were then reared for another 10 generations and assayed at each generation for ME attraction. In those assays, there was a gradual decrease in non-responders from *ca.* 35% to only 10% after the 7th generation. As pure isolines of non-ME-responding males were not attained, instead non-responders of those two lines in the 10th generation were used as sires to initiate the two

reduced-ME-responding lines. Further when NR flies were evaluated for their mating competitiveness, there was no significant difference of the number of copulations between NR and control males. Third, attraction of NR *B. dorsalis* males to semiochemicals revealed that those males demonstrated non-significant attraction to BCP and ZN compared with higher and significant attraction with control males. Fourth, the effects of semiochemical consumption by NR males and non-ME-fed males on their mating success was further evaluated. Wind tunnel and cage bioassays results demonstrated that NR males fed on BCP or ZN was more attractive to, and copulated earlier with virgin conspecific females compared with control unfed males. Semi-natural field cage studies also showed significantly higher copulation of BCP-fed NR males compared to BCP-deprived males whilst no significant difference was observed when ZN was offered to those males. These findings suggest that in the absence of pure non-ME-responding lines of *B. dorsalis*, BCP and ZN are potential semiochemical compounds that can be used to improve the mating performance of sterile reduced-ME-responding males in SIT trials.

Keywords: *Batrocera dorsalis*, Non -methyl eugenol- attracted, zingerone, beta-caryophyllene

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBANGUNAN LALAT BUAH JANTAN ORIENTAL, *BACTROCERA DORSALIS* (HENDEL) (DIPTERA: TEPHRITIDAE) BUKAN TERTARIK KEPADA METIL EUGENOL DITAMBAH SEMIOKIMIA

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Lalat buah Oriental, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) merupakan salah satu daripada perosak buah-buahan di dunia yang serius dan invasif. Kawalan serangga ini banyak bergantung kepada teknik penghapusan jantan (MAT) dan teknik pemandulan serangga (SIT). Penggunaan gabungan MAT dan SIT menawarkan kawalan yang sangat baik terhadap *B. dorsalis*. Walau bagaimanapun, isu dengan lalat jantan steril yang terbunuh akibat tertarik kepada perangkap MAT tersebut telah mengurangkan keberkesanan kedua-dua teknik tersebut. Walau bagaimanapun, ketersediaan *B. dorsalis* jantan yang bukan tertarik kepada ME akan membolehkan penggunaan MAT dan SIT secara serentak. Objektif kajian ini adalah untuk mewujudkan titisan domestik *B. dorsalis* yang disaring untuk bukan tertarik kepada ME, untuk menentukan ekspresi fenotip bagi bukan tertarik kepada ME, untuk menilai penarikan lalat jantan yang bukan tertarik kepada ME terhadap zingeron (ZN) dan β–kariofilena (BCP) dan untuk menilai kesan pemakanan bahan semiokimia tersebut oleh lalat jantan terhadap kejayaaan pengawanan mereka. Justeru, sebagai sebahagian daripada bukti konsep berskala makmal, kemungkinan pembangunan lalat jantan *B. dorsalis* bukan tertarik kepada ME (Lalat NR) dan ditambahkan dengan pendedahan kepada bahan semiokimia seperti ZN dan BCP untuk pengawanan yang dipertingkatkan berbanding lalat jantan liar telah diterokai. Pertama, titisan domestik koloni *B. dorsalis* yang dipelihara daripada larva dalam buah *Syzygium* sp. yang rosak, telah ditubuhkan dan disaring untuk bukan tertarik kepada ME. Lalat tersebut dikumpul dari empat lokasi berbeza dan lalat jantan tersebut didedahkan kepada ME berturut-turut selama 9 hari pada waktu pagi dengan selang 24 h di antara setiap hari pendedahan. Ketiadaan penarikan lalat jantan kepada ME ditakrifkan sebagai ketiadaan penarikan dalam sekurang-kurangnya dua pendedahan berturut-turut. Dua titisan berasingan lalat NR kepada ME daripada lokasi berlainan telah dit ubuhkan.

Kedua, ekspresi fenotip bukan tindak balas ME telah ditentukan dengan menilai tindak balas jantan terhadap bahan penarik dan daya saing mengawan lalat jantan NR kepada ME. Dua titisan NR kemudiannya dipelihara untuk 10 generasi lagi dan diuji pada setiap generasi untuk tertarik terhadap ME. Dalam ujian tersebut, terdapat penurunan beransur-ansur dalam bilangan yang bukan tertarik kepada ME, daripada 35% hingga hanya 10% selepas generasi ke-7. Memandangkan isotitisan jantan yang bukan tertarik kepada ME tidak dicapai, sebaliknya lalat yang bukan tertarik kepada ME daripada dua titisan dalam generasi ke-10 telah digunakan sebagai induk untuk memulakan dua titisan terkurang penarikan terhadap ME. Justeru, apabila lalat NR dinilai untuk keupayaan mengawan mereka, tidak terdapat perbezaan signifikan di antara bilangan yang bersanggama di antara lalat NR dan lalat kawalan. Ketiga, telah didapati penarikan lalat NR terhadap BCP dan ZN yang tidak berbeza secara signifikan tetapi lebih tinggi dan signifikan berbanding lalat kawalan. Keempat, kesan pemakanan bahan semiokimia oleh lalat jantan NR dan lalat jantan yang tidak memakan ME terhadap kejayaan mengawan mereka turut dinilai. Kajian terowong angin dan bioasai sangkar menunjukkan bahawa lalat jantan NR yang telah memakan BCP atau ZN adalah lebih menarik kepada lalat betina dara konspesifik dan bersanggama lebih awal berbanding lalat jantan kawalan yang tidak diberi makan ME. Kajian sangkar lapangan separa asli juga menunjukkan persanggamaan yang lebih tinggi secara signifikan bagi lalat jantan NR yang diberi makan BCP berbanding lalat jantan yang tidak diberi makan BCP. Malah, tiada perbezaan yang ketara diperhatikan apabila ZN diberikan kepada lalat jantan tersebut. Penemuan ini mencadangkan bahawa dalam ketiadaan titisan tulen bukan-tertarik- ME, BCP dan ZN adalah sebatian semiokimia yang berpotensi untuk digunakan bagi meningkatkan prestasi pengawanan lalat jantan mandul yang terkurang tertarik terhadap ME dalam dalam kajian SIT.

Kata Kunci: *Batrocera dorsalis*, bukan metil eugenol-tertarik, zingeron, β– kariofilena

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AW-PM	Area Wide-Pest Management
BCP	β-caryophyllene
DAE	Days after emergence
GC-MS	Gas Chromatography-Mass Spectrometry
MAT	Male Annihilation Technique
ME	Methyl eugenol
NR	ME-reduced responding males
SE	Standard error
SPSS	Statistical Soft Wear for Social Sciences
SIT	Sterile Insect Technique
UPM	Universiti Putra Malaysia
ZN	Zingerone

CHAPTER 1

INTRODUCTION

The Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is a notorious pest of agriculture in the tropical and subtropical regions of the world. Females of this species lay eggs and larvae feed upon many commercial fruits such as papaya (*Carica papaya* L.), guava (*Psidium guava* L.) and mango (*Mangifera indica* L.) causing significant yield losses (CABI, 2019). Along with a combination of wide-host range with higher fecundity (Huang & Chi, 2014), dispersal ability (Froerer *et al*., 2010), and capability to displace native fruit fly species (Allwood & Drew,1997; Reitz & Trumble, 2002; Lux *et al*., 2003), *B. dorsalis* have now been recorded in 75 countries (Zeng *et al*., 2019). Due to its invasive nature and negative impacts on agriculture, strict phytosanitary measures and large-scale surveillance programmes to detect *B. dorsalis* have led to implementation of suppression or eradication efforts (Alvarez *et al*., 2016; IPPC, 2017; Steck *et al*., 2019).

Several control tactics such as use of protein baits, soil drenching, fruit destruction, biocontrol agents, insecticides, Male Annihilation Technique (MAT) and Sterile Insect Technique (SIT) have been using singly or in combination to manage *B. dorsalis* (Vargas *et al*., 2015; Suckling *et al*., 2014). For example, MAT, which involves applying a mixture of highly potent male lure, methyl eugenol (ME), and an insecticide, significantly reduces the wild male population (Vargas *et al*., 2014a). MAT is the most frequently applied stand-alone eradication tool against *B. dorsalis*, having demonstrated several successes (Suckling *et al.*, 2014). SIT involves releasing many irradiated individuals of the target species, which allows mating with wild females resulting in no viable offspring produced, leading to a decline in the pest population (Knipling *et al*., 1955). Compared with other fruit fly species such as *Ceratitis capitata* and *Anastrepha ludens*, SIT has not been widely used yet against *B. dorsalis* (Suckling *et al*., 2014). However, SIT programmes are more effective and less costly if the density of wild males is decreased before the release of sterile males as this could reduce the number of sterile insects that need to be reared and released (Khan *et al*., 2017). Therefore, the use of MAT before SIT necessitates an increase in overflooding ratio (sterile male: wild male) of *B. dorsalis* but incurring high factory production costs of mass-reared sterile males. However, several instances have reported successful eradication *of B. dorsalis* through sequential application of MAT followed by SIT (Steiner *et al*., 1970; Habu *et al*., 1984).

Modelling studies have suggested that if MAT and SIT are used simultaneously, the release of sterile males can be significantly reduced thereby reducing production costs of those mass-reared sterile flies (Barclay *et al.*, 2014). However, that is contingent upon more wild males than sterile males are killed. In practice, MAT and SIT are used sequentially rather than simultaneously in managing *B. dorsalis*. There is also the possibility that sterile males continue to be attracted to, and killed in MAT bait stations due to longer periods of ME attractancy and toxicant efficacy in the field though there is a considerable time gap between SIT and MAT application.

The existence of males with ME-insensitive phenotypes was discovered during *B. dorsalis* eradication programme of Kikai Island in Japan (Ito & Iwahashi, 1974). Further, it was reported that the responsiveness of *B. dorsalis* to ME is decreased after two generations of selection in laboratory conditions. Further, cage studies from Hawaii, USA and China stated that non-ME-responsiveness could be attained via artificial selection i.e., 22 - 32% between 2-12 generations (Shelly, 1997) and 28.0 – 29.3% between 2-5 generations (Liu *et al*., 2017), respectively. Finally, Chen *et al.* (2018) reported that the male *B. dorsalis* non-responsiveness to ME was 1.7–1.9% in laboratory-reared and 3.4–4.3% in field strains in Taiwan. These findings suggest the possibility that non- ME responsiveness in male *B. dorsalis* can be developed as a possible strategy to overcome the problems of simultaneous application of SIT and MAT for control of *B. dorsalis*. Nevertheless, the above findings discussed the evolution of non-ME-responding flies via artificial selection involving twice exposure to ME in each generation. To date, there has been no reports on attaining non-ME-responsiveness within a single generation with multiple exposures to ME although it is certainly more time and cost-effective. Further, there is no information particularly on the mating success of those males particularly if they were to be developed as sterile males for simultaneous MAT and SIT application.

Pre-release feeding of male lures by sterile males have been shown to improve their mating competitiveness, thus increasing SIT's efficiency (Shelly *et al*., 2010; Shelly, 2020). This follows previous works that demonstrated (1) males of *B. dorsalis* gaining mating advantage following consumption of ME (Shelly & Dewire, 1994, Tan & Nishida, 1996), (2) ME-fed males that are significantly more attractive to conspecific females (Hee & Tan, 1998) and (3) bio-transforming ME after feeding on it, to sex pheromone compounds that are stored in rectal glands and emitted at dusk during mating (Nishida *et al*., 1988; Tan & Nishida,1996, Hee & Tan, 2004). Besides ME, compounds such as zingerone (ZN) and β-Caryophyllene (BCP) have also been shown to increase sexual signalling and mating competitiveness of other male dacine fruit flies such as melon fly, *Zeugodacus cucurbitae* (Coquilett) (Khoo & Tan, 2000) and guava fruit fly, *B. correcta* (Bezzi) (Wee *et al*., 2018 a) respectively. Both of those compounds are also known to be male lures of *B. dorsalis* (Tan & Nishida, 2000; Hee, 2016). This information suggests that possibility that consumption of those male lures by non-ME-responding males of *B. dorsalis* may confer them mating advantage thereby making up for the absent of advantages gained from consumption of ME.

Therefore, this study aims to develop a laboratory-scale proof-of-concept using non-ME-responding males supplemented with exposure to semiochemicals such as ZN and BCP for improved mating. A systematic investigation consisting four main objectives was undertaken as follows.

1. To establish domesticated lines of *B. dorsalis* colonies that reared from larvae in infested *Syzygium* sp. fruits and screening the males for non-ME responsiveness (NR) for initiate NR lines.
2. To ascertain the phenotypic expression of non-ME-responsiveness by assessing lure response and mating competitiveness of NR males.
3. To evaluate the attraction of NR males to zingerone and β–caryophyllene.

4. To evaluate zingerone & β–caryophyllene consumption by NR males and non-ME-fed males over mating success and female attraction.

Finally, the study outcomes provide the baseline data on behavioural aspects of NR males of *Bactrocera dorsalis* and their response to supplementary Semiochemicals, and the knowledge could be used to re-furbish the current fruit fly pest management strategies.

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