DEVELOPMENT AND MECHANISMS OF RESISTANCE IN PEACH FRUIT FLY *Bactrocera zonata* (SAUNDERS) TO MALATHION, LAMBDA-CYHALOTHRIN AND SPINOSAD

By

HUSSEIN MOHAMED NASOURI
B.Sc. Agric. Sci. (Plant Protection), Fac. Agric., Aleppo Univ., 2007
M.Sc. Agric. Sci. (Pesticides), Fac. Agric., Cairo Univ., 2012

THESIS
Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences
(Pesticides)

Department of Economic Entomology and Pesticides
Faculty of Agriculture
Cairo University
EGYPT

2017

APPROVAL SHEET

DEVELOPMENT AND MECHANISMS OF RESISTANCE IN PEACH FRUIT FLY *Bactrocera zonata* (SAUNDERS) TO MALATHION, LAMBDA-CYHALOTHRIN AND SPINOSAD

Ph.D. Thesis
In
Agric. Sci. (Pesticides)

By

HUSSEIN MOHAMED NASOURI
B.Sc. Agric. Sci. (Plant Protection), Fac. Agric., Aleppo Univ., 2007
M.Sc. Agric. Sci. (Pesticides), Fac. Agric., Cairo Univ., 2012

APPROVAL COMMITTEE

Dr. FAHMY AHMED MOHAMED KASSEM…………..……
Professor of Pesticides, Fac. Agric., Alexandria University

Dr. MOHAMED ABDEL-HADY KANDIL ….…………………
Professor of Pesticides, Fac. Agric., Cairo University

Dr. HANY MAHMOD ASHOUR BADAWY………………………...
Professor of Pesticides, Fac. Agric., Cairo University

Dr. DALIA AHMED ABD-EL-SALAM BARAKAT…………..
Professor of Pesticides, Fac. Agric., Cairo University

Date: / /

SUPERVISION SHEET

DEVELOPMENT AND MECHANISMS OF RESISTANCE IN PEACH FRUIT FLY *Bactrocera zonata* (SAUNDERS) TO MALATHION, LAMBDA-CYHALOTHRIN AND SPINOSAD

Ph.D. Thesis
In
Agric. Sci. (Pesticides)

By

HUSSEIN MOHAMED NASOURI
B.Sc. Agric. Sci. (Plant Protection), Fac. Agric., Aleppo Univ., 2007
M.Sc. Agric. Sci. (Pesticides), Fac. Agric., Cairo Univ., 2012

SUPERVISION COMMITTEE

Dr. HANY MAHMOD ASHOUR BADAWY
Professor of Pesticides, Fac. Agric., Cairo University

Dr. DALIA AHMED ABD-EL-SALAM BARAKAT
Professor of Pesticides, Fac. Agric., Cairo University

Dr. EMAN MOHAMED MOUSTAFA ALI RADWAN
Head Research of Pesticides, Agricultural Research Center

Name of the Candidate: Hussein Mohamed Nasouri **Degree:** Ph.D.
Title of Thesis: Development and Mechanisms of Resistance in Peach Fruit Fly *Bactrocera zonata* (Saunders) to Malathion, Lambda- Cyhalothrin and Spinosad.
Supervisors: Dr. Hany Mahmod Ashour Badawy
Dr. Dalia Ahmed Abd-El-Salam Barakat
Dr. Eman Mohamed Moustafa Ali Radwan
Department: Economic Entomology and Pesticides **Branch:** Pesticides
Approval: / /

ABSTRACT

The first part of the present study was undertaken to investigate the potential of peach fruit fly, *Bactrocera zonata*, to develop resistance to malathion, lambda-cyhalothrin and spinosad under laboratory conditions and the possible mechanisms of resistance. Three resistant strains were established; the malathion-resistant strain (M-R) (Resistance ratio (RR): 52-fold after eight generations of selection), the lambda-cyhalothrin-resistant strain (L-R) (RR: 12-fold after six generations of selection) and the spinosad-resistant (S-R) (RR <3-fold after six generations of selection). The L-R and S-R strains did not show cross-resistance to malathion. The selected strains were more tolerant to methomyl and deltamethrin, and more susceptible to dimethoate, lambada-cyhalothrin and spinosad. Piperonyl butoxide (PBO) had no synergistic effects for malathion and spinosad, while it increased the toxicity of lambda-cyhalothrin in both susceptible and L-R strains; and the synergistic effect was higher in L-R strain. Biochemical analysis revealed that esterase activity in the M-R strain was higher than that in the susceptible strain; these differences were significant in the eighth generation and in females of the sixth generation. There were no significant differences in esterase activity between the L-R and S-R strains and the susceptible strain. No significant differences in acetylcholinesterase (AChE) activity were found among males of all studied strains. However, significant differences were found between females of the third generation of the M-R and S-R strains and females of the susceptible strain. Glutathione-S-transferase (GST) activity was higher in all resistant strains than in the susceptible strain. The highest significant activity was recorded in the eighth generation of M-R. Cytochrome P450 activity in females of the third and eighth generations of the M-R strain and the third generation of S-R strain was significantly lower than that in the susceptible strain. Activity in females of the sixth generation of the L-R strain was significantly higher than that of susceptible strain. Sequencing study of *B. zonata* AChE cDNA revealed that the two mutations I214V and G488S, which are responsible for acetylcholinesterase insensitivity in some *Bactrocera* species, were missing in the M-R strain.

The second part of this study was undertaken to evaluate the efficacy of cover spray of Malathion Adwia 57% EC and Halothrin N 5% EC and bait spray of Conserve 0.024% CB against fruit flies in guava orchard in El-Beheira Governorate, Egypt. Throughout the experiment, peach fruit fly was absent from ammonium acetate traps and samples of infested fruits. Only the Mediterranean fruit fly was found. The cover spray treatment of Halothrin N 5% EC was the most effective with the lowest percentage of infested fruits and the highest reduction in infestation. The cover spray treatment of Malathion Adwia 57% EC was the least effective with the highest percentage of infestation and the lowest reduction in infestation. The bait spray of Conserve 0.024% CB did not provide sufficient protection against fruit fly infestation, and caused 54.4% reduction in infestation during the experimental period compared to 46.9 and 68.2% in the treatment of Malathion Adwia 57% EC and Halothrin N 5% EC, respectively.

Key words: Insecticide resistance, *Bactrocera zonata,* malathion, lambda-cyhalothrin, spinosad, cross-resistance, Piperonyl butoxide, detoxification enzymes, mutations, AChE cDNA, field efficacy, cover spray, bait spray.

DEDICATION

This work is dedicated to my beloved wife Heba and my adorable daughter Elena, Also, this work is dedicated to my Father, Mother and Brothers for their support and encouragement.

ACKNOWLEDGEMENT

I wish to express my sincere thanks, deepest gratitude and appreciation to ***Dr. Hany Mahmod Ashour Badawy*** *and* ***Dr. Dalia Ahmed Abd-El-Salam Barakat,*** *Professors of Pesticides, Faculty of Agriculture, Cairo University, and* ***Dr. Eman Mohamed Moustafa Ali Radwan,*** *Head research and Head of the Department of Population Toxicity, Central Agricultural Pesticides Laboratory, Agricultural Research Center, for their valuable advice, continuous encouragement, cooperation, and kind assistance, supervision, critical reading and correction of the manuscript, constructive criticism and efforts to fulfill this work.*

Grateful appreciation is also extended to all staff members of the Economic Entomology and Pesticides Department, Faculty of Agriculture, Cairo University.

Special deep appreciation is given to my beloved wife and daughter, my father, my mother, and my brothers. Also, I feel deeply grateful to my dear country Syria and my second country Egypt.

CONTENTS

CONTENTS (continued)

INTRODUCTION

The fruit fly family Tephritidae contains some of the most damaging fruit pests in the world. Among them, the peach fruit fly, *Bactrocera zonata* (Saunders), which is native to South & South-East Asia and then has spread to Pakistan, Arabian Peninsula and Egypt. Its presence has also been recorded later in south Iran and Lebanon (European and Mediterranean Plant Protection Organization, 2010). This fly has been recorded on over 50 cultivated and wild plant species mainly with fleshy fruits. The main hosts are guava, mango and peach. Secondary hosts include apricot, fig and citrus (Hosni *et al.*, 2013). Female flies lay eggs in the fruits and the larvae feed on the pulp. Subsequently, fruits became vulnerable to secondary bacterial and fungal infections and infested fruits drop down (Mosleh *et al.*, 2011).

In Egypt, the Mediterranean fruit fly, *Ceratitis capitata,* and the peach fruit fly, *Bactrocera zonata,* are the most destructive insect pests of fruits. The Mediterranean fruit fly is the key-pest of orange fruits, while the peach fruit fly is the key-pest at the period of occurrence of guava, mango, and peach fruits (El-Gendy and Nassar, 2014). The Mediterranean fruit fly is well established in Egypt and has the ability to tolerate cold climates better than most other species of fruit flies (Steck, 2006). The peach fruit fly was detected in Egypt in 1914 in an intercepted consignment from India in Port Said (Efflatoun, 1924). It disappeared for a long period, and then in 1993, it was detected from guava samples in Qalyubia and Faiyum Governorates. Also, it was found in Alexandria Governorate (Agami), where fig is widely distributed and in Giza Governorate, where different horticultural trees were cultivated. By 1995, the insect was found in further fruit producing Governorates and then, in 1997 it was distributed throughout Egypt (De Meyer *et al*., 2007).

B. zonata is known in India and South-East Asia as a serious pest of tropical and subtropical fruits. Economic impacts may result from costly eradication measures, quarantine restrictions imposed by important domestic and foreign import markets, and from direct yield losses as a result of infested fruit. It is one of the three most destructive fruit flies in India, causing crop losses of 25 to 100% in peach, apricot, guava and figs (Bakri, 2008). In Pakistan, it causes losses of 3 to 100 % in different fruits, where the damage in guava fruits reached to 25-50% (Siddiqui *et al*., 2003 and Kakar *et al*., 2014). In Egypt, *B. zonata* has caused an estimated 190 million Euro damage a year (European and Mediterranean Plant Protection Organization, 2005). Also, the infestation of apricot and citrus with *B. zonata* was higher than that with *Ceratitis capitata* (Saafan *et al*., 2005)**.**

Major control and eradication programs have been developed in many parts of the world to combat fruit flies. The array of control methods includes insecticides spray to foliage and soil, bait-sprays, male annihilation techniques, releases of sterilized flies and parasitoids, and cultural controls (Vargas *et al.*, 2015). The use of insecticides applied as cover sprays to the affected crops to prevent fruit fly damage is common practice in many African and Asian countries (FAO, 1986). The use of cover sprays in the Arab countries especially with the organophosphate insecticides malathion or dimethoate against fruit flies has been practiced for many years, and is still considered a very effective and relatively cheap control method for fruit fly (Lysandrou, 2009). In the cover spray system, the insecticides are usually applied on a calendar basis beginning at the time the respective fruits becomes susceptible to oviposition and continued at weekly intervals until about 1-2 weeks before the fruits are harvested (Vijaysegaran, 1993). Advantages of insecticide cover sprays are that they are affordable, convenient and provide a high level of protection against fruit fly infestation with consistent results (Allwood, 1997). However, when misused, cover spray can lead to a number of problems such as increasing the risk of insecticide resistance, high level of insecticide residues on harvested fruits, and harmful effects on non-target organisms. Bait spray was first reported as effective control method against fruit fly by Steiner (1952). Since then, protein bait sprays have become a major method of suppressing or eradicating fruit fly populations in many parts of the world. Bait sprays work on the principle that mainly female Tephritid fruit flies are strongly attracted to a protein source from which ammonia emanates, and ingest a lethal dose of insecticide together with the protein. Bait sprays have the advantage over cover sprays in that they can be applied as a spot treatment so that the flies are attracted to the insecticide and there is minimal impact on natural enemies (European and Mediterranean Plant Protection Organization, 2005). Also, it reduces the amount of pesticide needed for fruit fly control (Roessler, 1989 and Prokopy *et al.*, 1992). On the other hand, bait sprays are not widely adopted by farmer in developing countries because protein baits are expensive and inaccessible to a large number of fruit growers where it is imported from foreign sources, also, many growers probably apply bait sprays in high volumes, much similar to insecticide cover sprays, thus providing little additional benefit (Vijaysegaran, 1993).

A wide range of insecticides are used in fruit fly control progams. Malathion is one of the most important insecticides; it has been used extensively in fruit fly control programs. Malathion was used in fruit fly control as early as 1954 (Steiner, 1954) and it became the additive in protein hydrolysate baits due to its low mammalian toxicity, affordable price, and low levels of fruit fly resistance (Roessler, 1989). However, during the twenty first century there has been a trend to move away from control with organophosphate insecticides (*e.g.*, malathion, diazinon, and naled) towards reduced risk insecticide treatments (Vargas *et al.*, 2015); and since the withdrawal of malathion in the European Union in 2009, lambda-cyhalothrin and spinosad have become the most widely used insecticides for the control of fruit flies, although lufenuron, etofenprox and methyl-chlorpyrifos have also been used (Arouri *et al.*, 2015). Spinosad bait treatment is considered an effective and environmentally safe alternative to conventional bait sprays that incorporate organophosphates as killing agents (Mangan *et al.*, 2006). Spinosad has low mammalian toxicity and reduced environmental impact on natural enemies (Vargas *et al.*, 2015) and it has been approved for organic fruit and vegetable production (Dow AgroSciences, 2009).

Pesticides resistance is an increasingly urgent worldwide problem. Resistance to one or more pesticides has been reported in more than 440 species of insects and mites (Tabashnik and Roush, 1990). There were more than 7747 cases of resistance with more than 331 insecticide compounds involved according to the database developed at Michigan State University in 2008. From the estimated 10,000 arthropod pests, 553 species were reported with resistance to insecticides. Approximately 40% of resistant arthropods are medical pests, and close to 60% are agricultural pests (Whalon *et al.*, 2008 and Onstad, 2014). Very few indications of insecticide resistance were reported for Tephritidae fruit flies and insecticide resistance had never become a practical problem despite widespread applications of a variety of different insecticides (Wood, 1986). Among the factors that may have a delaying effect on the evolution of resistance in fruit flies are the natural movement of flies between treated and untreated trees or areas and broad range of alternative hosts, thus escaping continuous exposure to chemicals. Since 2000, several reports have documented insecticide resistance in economically important species of fruit flies, which in turn is becoming a problem for effective control. An organophosphate-resistant strain of the olive fruit fly, *Bactrocera oleae*, was obtained by laboratory selection with dimethoate (Vontas *et al.*, 2001). Ten resistant lines of the oriental fruit flies, *Bactrocera dorsalis*, were selected separately to six organophosphates (naled, trichlorfon, fenitrothion, fenthion, formothion, and malathion), one carbamate (methomyl), and three pyrethroids (cyfluthrin, cypermethrin, and fenvalerate) (Hsu *et al.*, 2004). Resistance was reported in Spanish field populations of *Ceratitis capitata* to malathion (Magaña *et al.*, 2007). In Egypt, Radwan (2012) found that a field population of *B. zonata* was highly resistant to malathion. Also, in Pakistan, Nadeem *et al*. (2014) documented moderate level of malathion resistance in some field population of *B. zonata*.

Studying the mechanisms by which pests become resistant to pesticides is extremely important. Knowledge of resistance can provide fundamental insights into evolution, genetics, physiology, and ecology (Tabashnik and Roush, 1990). There are three major classes of mechanisms of resistance to insecticides in insects. The first class is target site insensitivity which is allelic variation in the expression of target proteins with modified insecticide binding sites, *e.g.*, acetylcholinesterase insensitivity towards organophosphates and carbamates, voltage-gated sodium channel mutations responsible for knockdown resistance to pyrethroids, and a serine to alanine point mutation (rdl gene) in the *γ*-aminobutyric acid (GABA)-gated chloride channel ($GABA_A$-R) at the endosulfan/fipronil/dieldrin binding site (Zhu and Clark, 1997; Bloomquist, 2001; Gunning and Moores, 2001 and Siegfried and Scharf, 2001). The second - and often most important - class of resistance mechanisms in insect pest species is metabolic degradation involving detoxification enzymes such as microsomal cytochrome P-450 dependent monooxygenases, esterases, and glutathione S-transferases (Field *et al*., 2001 and Scott, 2001). The third, least important mechanism is an altered composition of cuticular waxes which affects penetration of toxicants. Reduced penetration of insecticides through the insect cuticle has often been described as a contributing factor, in combination with target site insensitivity or metabolic detoxification (or both), rather than functioning as a major mechanism on its own (Oppenoorth, 1985). Most of the mechanisms mentioned above affect in many cases the efficacy of more than one class of insecticides, *i.e*., constant selection pressure to one chemical class could to a greater or lesser extent confer cross resistance to compounds from other chemical classes (Oppenoorth, 1985 and Soderlund, 1997).

The present study aimed to investigate the following items:

1. The development of resistance to malathion, lambda-cyhalothrin and spinosad in the peach fruit fly.
2. The pattern of cross-resistance to other insecticides in the resistant strains.
3. The synergistic effect of piperonyl butoxide (PBO) on the tested insecticides against the susceptible and resistant strains.
4. The potential role of esterase, acetylcholinesterase (AChE), glutathione-S-transferase (GST) and cytochrome P450 in the development of resistance to malathion, lambda-cyhalothrin and spinosad in the three selected laboratory strains of peach fruit fly.
5. The sequence of *B. zonata* AChE cDNA in order to detect the presence of two mutations I214V (isoleucine to valine substitution) and G488S (glycine to serine substitution), which are responsible for acetylcholinesterase insensitivity in some organophosphate-resistant *Bactrocera* species.