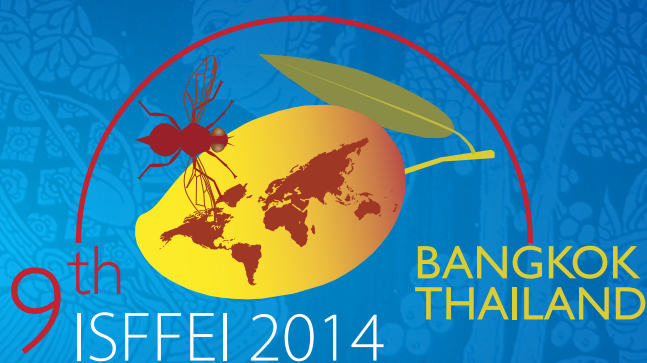


The Ninth  
International Symposium on  
Fruit Flies of Economic  
Importance (9<sup>th</sup>ISFFEI)



# PROCEEDINGS



12-16 May 2014  
Bangkok, THAILAND

EDITED BY

- Beatriz Sabater-Muñoz
- Teresa Vera
- Rui Pereira
- Watchreeporn Orankanok







# **Proceedings of the 9<sup>th</sup> International Symposium on Fruit Flies of Economic Importance**



## **2016**

**Edited by:**

**Beatriz Sabater-Muñoz**

**Teresa Vera**

**Rui Pereira**

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**First Edition 2016**

**“Proceedings of the ninth international symposium on fruit flies of economic importance”**

**ISBN 978-616-358-207-2**

**(C) Edited by Beatriz Sabater-Muñoz, Teresa Vera, Rui Pereira and Watchreeporn Orankanok**

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Participants' group picture at the 9th ISFFEI venue in Bangkok.

## **FOREWORD**

The International Symposium on Fruit Flies of Economic Importance (ISFFEI), an event convened once every four years, serves as forum for scientists and stakeholders all across the world to share know-how, new techniques and technologies, control methods, among others related to Tephritids control. This forum is also a platform for initiation of young scientist and technologists, and for the establishment of new bonds and cooperation between participants.

This symposium series started in 1982 in Athens (Greece), and since then, it achieved nine successful meetings. In 2010, the International Fruit Fly Steering Committee (ISFFSC), a group of fruit fly specialists from around the globe, in charge of selecting the new venues and local Organizing Committees (OC), increased its numbers till 18-20 members to cover almost all geographical representatives, including the outgoing Chairperson, the outgoing and incoming representatives of the OC, and the Chairpersons of related working groups as the TEAM (Tephritids workers of Europe, Africa and the Middle East) and TWWH (Tephritids workers of the Western Hemisphere). In 2014, the Chairperson of TAAO (Tephritids workers of Asia, Australia and Oceania) was also included in this international Steering Committee.

The rising demand of fresh vegetables and fruits, along the growing human populations, and limited crop areas is a challenge that should be faced by all. The climatic conditions in tropical and subtropical countries represent an opportunity to cover this lucrative market, but, also is a drawback, due to the presence of many Tephritid species menacing all these crops and posing a trade barrier. Fortunately, as explained, the rise in demand, and production, is being also supported by an increase in Tephritid workers communities. The economical support of local governments, and the involvement of growers, to find environmental and sustainable production still represent a challenge, but readers could find within the present book, some of the solutions proposed in several countries. The expansion of crops with overlapping production of putative fruit fly hosts all around the year, still deserves control program implementation. We expect that in the next few years all the Tephritid workers strength the connections started in Thailand, to find solutions based on the previous knowledge here presented and meet again in Tapachula, Mexico, our next venue.

Last but not least, we wish to emphasize that this community is an evolving team. Knowledge exchange and discussion has always been encouraged in our symposiums both by the SC members and ISFFEI assistants. Since Thailand some of them are not with us anymore. It is our duty to transmit their enthusiasm to understand fruit fly biology and apply this knowledge to solve people's needs. This volume wishes to honor all of them, but particularly Serge Quilici (1955-2015), a SC member from La Reunion, France. Serge devoted his research to fruit fly behavior, invasive species, interspecific competition, host selection, semiochemicals and the role of plant volatiles in attracting female flies. People familiar with these topics will recognize his extensive and serious work and commitment with the fruit fly community. But above this, he was a great person. Always willing to assist you, if this was in his hands. We hope his heritage both as researcher and person remains in our memory.



**The International Fruit Fly Steering Committee (IFFSC)****2010 to 2014****IFFSC Chair:** Aldo Malavasi (Brazil)**IFFSC Members (in alphabetical order):**

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 Abraham Verghese (India)



Part of the 9th ISFFEI Organizing Committee



The 9th ISFFEI Steering Committee

## **EDITORS' PREFACE**

The 9<sup>th</sup> ISFFEI took place on Bangkok, Thailand, from 12 to 16 May 2014. Under the theme “Cordial strong bond beyond fruit fly work”, this symposium joined nearly 400 participants from 60 countries. Nearly 200 communications (oral and posters) distributed among nine different sessions, were presented.

The papers contained in this volume report the scientifically reviewed works presented at this symposium series. Keynote speakers, contributed oral and poster presentations authors were given the opportunity to submit a manuscript for publication.

Manuscripts had been reviewed by a panel of experts, members of the Fruit Flies Steering Committee and, finally by the Editors. Only those papers judged suitable for publication following the authors' considerations to the reviewers comments, appear in this volume.



## ACKNOWLEDGEMENTS

The editors of this volume on behalf of the 9th ISFFEI International Steering and Organizing Committees would like to acknowledge the economical support of the Government of Thailand, specially from the Ministry of Agriculture and Cooperatives, the Department of Agricultural Extension (DOAE), and the Thailand Convention and Exhibition Bureau (TCEB) along the private contribution of several international companies and even those of local fruit cooperatives, that allowed to develop the 9th ISFFEI in a pleasant atmosphere in Bangkok. In addition, we would like to acknowledge the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture which constantly supports these meetings.

In addition, we would like to acknowledge all the components of the local organizing committee, some of them in the picture here below.



The 9<sup>th</sup> ISFFEI Organizing Committee (partial), from DOAE, with traditional Thai dresses.





The 9<sup>th</sup> ISFFEI opening ceremony speakers



The 9<sup>th</sup> ISFFEI starting gong at the opening ceremony, with Dr. Jorge Hendrichs, Mr. Olan Pituck (Director-General of DOAE), Dr. Aldo Malavasi, and his excellency the Minister of Agriculture and Cooperatives.

The Editors acknowledge and appreciate the contribution of the reviewers listed below, except some that asked to keep their anonymity, by their significant contribution to improve the quality of this publication. Last but not least, we are grateful to Nathalie Ghazarian at Insect Pest Control Joint FAO/IAEA Division (Austria) for her help with the layout of this volume.

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## **Area-Wide & Action Programmes**

## **Fruit fly area-wide integrated control program in Thailand success or failure?**

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### **Abstract**

Two *Bactrocera* species, *Bactrocera dorsalis* (Hendel) and *Bactrocera correcta* (Bezzi) are classified as importance pest and constrained international fruit trade of Thailand. For more than two decades, Thailand with the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency (FAO/IAEA) support implemented the sterile insect technique (SIT) to suppress fruit fly populations. Since 1991, in collaboration with the Office of Atoms for Peace, irradiated pupae were weekly shipped and directly ground released in 720 ha of mango orchards, in Ratchaburi province. In 1995, a small mass-rearing facility with capacity to produce 40 million pupae per week was established. An Area-Wide Integrated Pest Management (AW-IPM) approach was accepted since 2001 and SIT was implemented in suppression strategies to control *B. dorsalis* and *B. correcta*. Sterile flies of both species were released over two areas of mango of 3,440 ha in Ratchaburi province and of 3,670 ha in Phichit province since 2003. The white thorax strain of *B. dorsalis* was developed by Thailand Institute of Nuclear Technology (TINT) in 2007 and sterile flies were released over 2,590 ha of tropical fruit plantation in Chanthaburi province. Other control methods were integrated in each area depending on community participatory decision. Fruit damage was reduced from over 80% to less than 3.6% in the last five years (2000 to 2004) in Ratchaburi. In Phichit, fruit infestation was reduced from 43% in 2002 to 15% in 2004 (two years after the suppression initiation). Exportation possibility has been opened for mango produced in these areas to some of the most stringent and lucrative markets. Few year later, after SIT was stopped in 2005, the infestation was increased to 17% in Ratchaburi, and with less sterile flies released in Pichit, the damage bounced back to 23-30%. For Chanthaburi province, average sterile to wild (S/W) ratio has been increased to 6.75 while flies per trap per day (FTD) were maintained at low levels, 0.67 in 2013 compared with 1.20 and 2.37, prior to the suppression activities. Various factors affected population suppression degree and inadequate budget has always been major constraint. However, currently the fruit fly control program has been emphasized as national agricultural strategy and the high executive administrator has considered it of importance.

**Keywords:** *Bactocera dorsalis*, *Bactocera correcta*, population suppression, sterile insect technique.

## Introduction

*Bactrocera dorsalis* (Hendel) and *B. correcta* (Bezzi) are classified as the major insect pests in Thailand and impose a significant cost on horticulture production, especially to a wide range of soft fruits. The economic loss in mango production is estimated in one hundred million dollars per year (Enkerlin, 2001). International trade barrier also contribute for these losses. Conventional control involves insecticide application, which increase the production costs (Enkerlin, 2001) and contributes to the increase of residue in fruits and environmental pollution. Bagging fruit can reduce damage but is labor intensive and sanitation and removal of infested or remained fruits is not widely practiced. Export of Thai fresh fruits requires post-harvest treatment prior to shipment to attractive fruit fly free markets, such as Japan and the United States of America (USA), reducing the benefits. All these, makes imperative the need of implementing a successful Area Wide-Integrated Pest Management (AW-IPM) program.

*Bactrocera dorsalis*, or the Oriental fruit fly, is considered a primary quarantine pest for many of Thailand's trading partners. It has been identified and recognized as the most important pest of Asia, Southeast Asia and Pacific (Mau and Matin 1992, White and Elson-Harris 1992, Sutantawong et al. 2004). The area of origin can be confidently placed in the Southeast China region facing the South China Sea (Wan et al. 2012). It has been introduced and established in some Pacific islands, such as the Hawaiian Islands around 1945 and Guam in 1947 (Waterhouse 1993, Gary 2007, Stephens 2007, Vargas et al. 2007, Schutze et al. 2012) and more recently in Africa in the Coast Province of Kenya (Drew et al. 2005).

*Bactrocera correcta* (Bezzi), although is polyphagous is often referred to as the guava fruit fly (White and Elson-Harris 1994), and is listed as a quarantine pest by most countries worldwide. This fly was first recorded in India in 1916 and is now distributed throughout most countries of Southeast Asia (White and Elson-Harris 1992, Wang 1996, Drew and Raghu 2002). It has a wide host range of tropical and subtropical fruits belonging to 30 plant families (Allwood et al. 1999, Maynard et al. 2004) and causes great losses on economically valuable fruits and vegetables in Vietnam and central to northern Thailand (White and Elson-Harris 1992, Drew and Raghu 2002).

Bearing in mind the economic impact of these two species in Thailand, the Office of Atoms for Peace (OAP) initiated a program against *B. dorsalis* in Chiang Mai province, north of Thailand in 1982 implementing the sterile insect technique (SIT) involving several activities. Sterile flies were released over a plum plantation in the Royal Ang Khang Research Station, Ang Khang Mountain located at 1,928 meters elevation. The SIT technology was transferred from OAP to the Department of Agricultural Extension (DOAE) in 1987. Since then, the Oriental fruit fly control program using SIT was expanded to other provinces in Thailand and implemented as suppression strategy of AW-IPM in 2001 (Enkerlin 2005). Adult surveillance was established and environmental-friendly control methods, such as male annihilation, bait application, orchards sanitation, removal of alternative, potential and wild host were also implemented. Initially, this was done in close collaboration with the International Atomic Energy Agency (IAEA) of the United Nations, Joint FAO/IAEA Division of Nuclear

Techniques in Food and Agriculture, OAP, Thailand Institute of Nuclear Technology (TINT), Department of Agriculture (DOA), Mahidol University (MU) and DOAE. Later, the support of governor office, local administrative organization and the private sector has helped the project move forward. The AW-IPM by using SIT has been effective in controlling fruit flies by reducing damage and has opened the possibility for export of fresh fruits produced in these selected pilot areas to some of the most stringent and lucrative markets, such as Japan and the USA. An economic feasibility study conducted in 2002 clearly showed that fruit fly control in Thailand using AW-IPM by integrating SIT could be expanded to other fruit production areas with significant economic returns (Knight, 2002).

The program was first focused in Phichit province since field operational costs were mainly covered by growers rather than the national government and in Chanthaburi province where sub-district administrative organization played an important role in involving the community. During the implementation of SIT for fruit fly control in Thailand, the program has faced certain difficulties such as deficient sanitary practices, by no removal of infested fruits and other non-technical issues like inadequate budget were a major constraint that caused high variability in sterile fly production. Currently, the fruit fly control program in Thailand is facing two main challenges. First, the project should be scaled-up from small area projects to a country-wide initiative. Second, the AW-IPM program should become self sufficient by encouraging full financial support from crop growers and stakeholders.

Here we describe activities and results of fruit fly control actions implemented during 2000-2014. These activities involved sterile fruit fly mass-rearing and release, surveillance and other control activities. We also provide the main results from two economic analyses and from research carried out to support the program.

### **Sterile Insect Technique**

The OAP has pioneered the SIT in Thailand at the Royal Ang Khang Research Station, Ang Khang Mountain, Chiang Mai province since 1982 and a pilot field trial using SIT was initiated in 1987. Since 2001, an AW-IPM approach has been accepted and implemented. So, for more than 2 decades, Thailand with FAO/IAEA support has conducted the SIT integrated with other environmental-friendly methods for fruit flies population suppression.

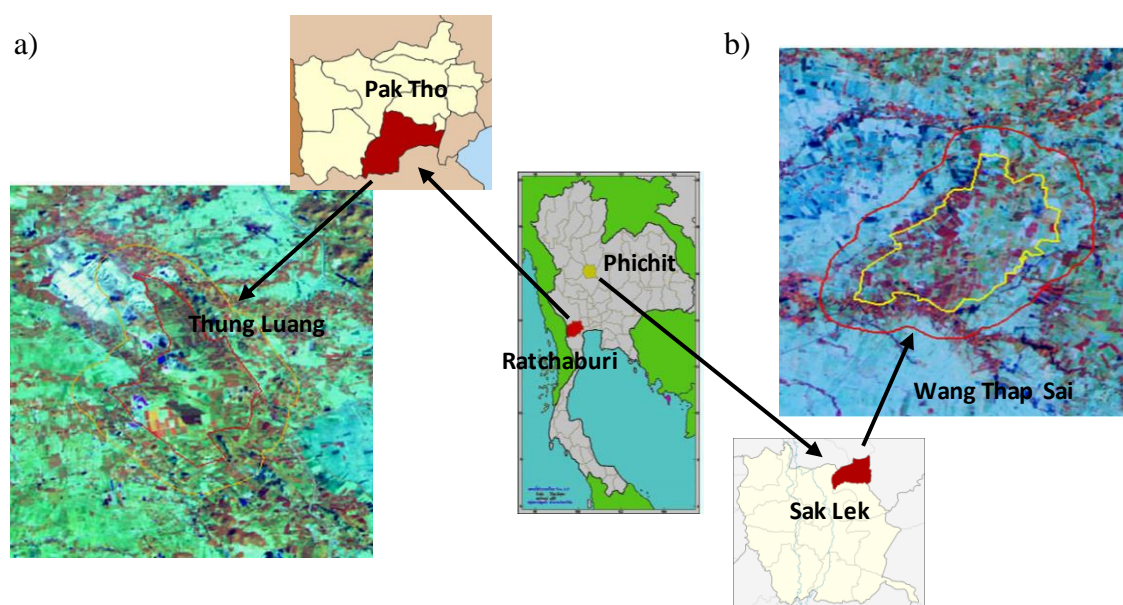
#### *Supression areas*

Given that mango (*Mangifera indica*), the traditional crop in Thailand with more than 352,000 ha cultivated throughout the country, was considered as the main crop for fruit fly control, the sterile releases were conducted in mango orchards.

The pilot field trial using SIT was initiated in two different low-land areas, where *B. dorsalis* was the dominant fruit fly species: the Thung Luang sub-district, Pak Tho district, Ratchaburi province (13°35'N, 99°83'E), 150 km southwest of Bangkok and Ban Chang district, Rayong province (12°41'N; 101°04'E), 170 km east of Bangkok. Sterile *B. dorsalis* were released over 720 ha of mango orchards in both areas. Tropical climatic conditions allow for a high diversity and continuity of fruit hosts making isolation of these orchards virtually impossible.



In 1994, DOAE extended the SIT programme to Pak Chong district, Nakhon Ratchasima province, 160 km northeast of Bangkok, in 720 ha of many kinds of tropical fruits. Since 2001, the AW-IPM approach has been implemented and due to the most appropriate geographical isolation, only the first SIT area of 720 ha mango orchards in Thung Luang sub-district, Pak Tho district, Ratchaburi province was selected and expanded to 1,340 ha as the first AW-IPM area using SIT for fruit fly population suppression in Thailand. Sterile *B. dorsalis* were released over mango and other fruit orchards as core area, and with 2,100 ha surrounded by areas under cultivation of field crops, paddy field and mountains as buffer zone (Fig. 1a). Twenty-two ground release points were fixed by GPS uniformly spread at distances of 1,000 meters in the release area.



**Fig. 1.** The AW-IPM pilot area using SIT for fruit fly control in Thailand. a) First pilot area located in Thung Luang sub-district, Pak Tho district, Ratchaburi province (1,340 ha of core area). b) Second pilot area located in Wang Thap Sai sub-district, Sak Lek district, Phichit province (1,670 ha of core area).

In 2002, *B. correcta* was reported as one of the most serious insect pest of commercial fruit crop in fruit fly control area. During 2003-2008, both sterile *B. dorsalis* and *B. correcta* were released in Thung Luang sub-district, Pak Tho district, Ratchaburi province and then releases were expanded to the second area in Wang Thap Sai sub-district, Sak Lek district, Phichit province. This second area, located at 16°43'N; 100°51'E, 450 km northwest of Bangkok, is a large mango plantation. The core area involved 1,670 ha and 2,000 ha surrounded by field crops and mountains were marked as buffer zone (Fig. 1b). Release sites were marked for better sterile flies distribution with a uniformly spread distance of 350 meters. The SIT, integrated with other environmentally-friendly control methods, such as male annihilation, bait application, orchards sanitation and removal of hosts, were implemented in a suppression strategy in both sites. Approximately 2,000 *B. dorsalis* and 1,000 *B. correcta* sterile flies per hectare were released during the critical months of fruit season (October to March). Meanwhile, trapping network system involving weekly inspection of methyl eugenol-baited Steiner traps as well as twice a month of fruit sampling were applied for adult and larval

surveillance in Trok Nong sub-district, Khlung district, Chantaburi province located at 12°27'17"N; 102°13'17"E, where marketable quality of mangosteen couldn't be exported to premium markets due to fruit flies. Due to grower requirement for SIT, a project using SIT for fruit fly control was implemented in this area during 2007-2012 by TINT. The 2,590 ha, with durian, mangosteen, and long kong, were designed as 1,570 ha core area and 1,020 ha buffer zone (Fig. 2).



**Fig. 2.** Third pilot area of suppression using SIT-AW-IPM for fruit flies control in Thailand in Trok Nong sub-district, Khlung district, Chantaburi province (1,570 ha of core area).

The sterile flies of white thorax strain of *B. dorsalis* which was developed by TINT were released and integrated with other control techniques. In 2013, this area was considered as the third pilot SIT-AWIPM area of DOAE, with growers' high intention to establish the first area of low pest prevalence for fruit fly in Thailand. Five million sterile *B. dorsalis* were released weekly over this pilot area. Sub-district Administrative Organization (SAO) participated with grower support on orchards sanitation, removal of wild hosts, mass-trapping, and larval and adult surveillance. In 2014, DOAE raises pest control to one of its important policies. Ten thousands fruit growers in 20 provinces were the focal point to improve fruit fly knowledge and control technologies.

### Mass-rearing, sterilization and release methods

In 1995, a small pilot fruit fly mass-rearing facility of DOAE was established in Thanyaburi district, Pathum Thani province, 34 km east of Bangkok with a capacity to produce 40 million sterile flies per week. The original objective was to mass-rear *B. dorsalis* and irradiated pupae were weekly transported to and directly released in the target area.

With IAEA support, many improvements were implemented over the past decade in the mass rearing facility. Plywood adult cages were completely replaced by stainless steel cages 40 x 180 x 175 cm (W x L x H) constructed of stainless steel covered with aluminum screen mesh (Fig. 3a, 3b) in August 2005. The existing medium modified larval artificial diet mixer was replaced with an industrial semi-automatic mixer (Fig. 3c, 3d). On ground larval popping trolley was modified to force the popped larvae dropped to underneath sawdust tray (Fig. 3e, 3f). Wooden frame pupae holding trays were substituted by stainless steel frame trays and iron racks for holding pupae trays were replaced by stainless steel racks (Fig. 3g, 3h). The aluminum pupae sifting machine was replaced by a stainless steel machine (Fig. 3i, 3j). The

rearing methodology was also improved. In July 2006 the age at which the pupae were sifted was changed from 24 hours after larvae collection to 24 hours before irradiation that is 3 days before emergence. In May 2007, irradiation methodology was definitely changed through hypoxia condition (Fig. 3k, 3l) and irradiation processes were improved in terms of: 1) fluorescent dye 2) packed and banded in polyethylene sausage 3) irradiated and 4) packed in container.



**Fig. 3** Equipments and irradiation methodology improvement (a) Plywood adult cage; (b) Stainless steel adult cage; (c) Medium modified larval artificial diet mixer; (d) Industrial semi-automatic mixer; (e) On ground larval popping trolley; (f) Modified larval popping trolley; (g) Wooden frame pupae holding tray and iron rack for holding pupae tray; (h) Stainless steel frame pupae holding tray and stainless steel rack for holding pupae tray; (i) Aluminum pupae sifting machine; (j) Stainless steel pupae sifting machine; (k) Oxygen condition irradiation. and (l) Hypoxia irradiation

After *B. correcta* was reported as a serious pest of the commercial fruit crop in the control area in 2002, both *B. dorsalis* and *B. correcta* were produced at the facility. One hundred thousand adult flies each of *B. dorsalis* and *B. correcta* were housed in rectangular stainless steel oviposition cages 40 x 180 x 175 cm (Fig. 4a) and maintained in separate rooms under  $25 \pm 1^\circ\text{C}$ , 65-70% R.H. and 12:12 D:L condition. Adults were fed *ad libitum* with an artificial diet and water (Fig. 4a). Eggging was done when females were 10–25 days old by placing lines of perforated bottle contained a sponge soaked with 10% guava juice for 24 hours (Fig. 4a). Bottles were removed once a day and rinsed with water to collect the eggs (Fig. 4b). Collected eggs were stored in semi-liquid agar (Fig. 4c) and if necessary preserved at  $18 \pm 2^\circ\text{C}$  for 1-3 days then seeded on a wheat bran based diet (Sutantawong et al. 2004) (Fig. 4d). A maximum of 18 seeded trays were placed as tower in a trolley and stored in incubation condition,  $28\text{--}32^\circ\text{C}$  in dark, for egg hatch period. Larvae were then transferred to the developing room,  $25 \pm 1^\circ\text{C}$ , 65-70% R.H. (Fig. 4e). At day 5<sup>th</sup>–8<sup>th</sup> after seeding, mature popped larvae were collected in the early morning ( $\approx 8.00$  h) and late afternoon ( $\approx 16.00$  h) in sawdust trays and



maintained in 20°C, dark room, for pupation. Pupae trays of different collection were alternately stored in 20±1 or 25±1°C room for delay or activate pupae age synchronized. Pupae were separated from sawdust 3 days before emergence (Fig. 4f) by two individual mechanical sifting devices. The day after separation, pupae were marked with 2.0 gram fluorescent dye powder per litre of pupae and were packed in polyethylene bags and were banded (Fig. 4g). Packed pupae were sterilized using gamma radiation at dose 90 Gy and 80 Gy for *B. dorsalis* and *B. correcta*, respectively, from a Co60 Gammacell 220 source within the mass-rearing facility (Fig. 4h). Irradiated pupae were kept in polystyrene containers with ice packs (Fig. 4i). A refrigerated truck was used for weekly shipments (Fig. 4j), and irradiated pupae were delivered directly to the emergence and holding facilities on the same day of irradiation. Pupae were incubated for emergence in plastic boxes at 26-27°C room temperature for 3-4 days (Fig. 4k) and sterile adult flies were ground released at fixed release sites in the field using pupal boxes (Fig. 4l).



**Fig. 4** Fruit fly mass-rearing, sterilization and release process; (a) Adult flies are housed in rectangular oviposition cages where female flies lay eggs into lines of perforated bottle contained a sponge soaked with 10% guava juice; (b) Eggng bottles are removed from the cages for egg collection; (c) The well mixed of eggs: agar and water at 1:6.5:2.5 ratio which are ready for seeding; (d) The mixtures of collected egg are seeded on a wheat



bran based diet; (e) The seeded trays are placed in a trolley and transferred to the initiation room and developing room, g) Pupae trays are stored at  $20\pm 1$  or  $25\pm 1^\circ\text{C}$  room for delay or activate of pupae age synchronized; (h) Fluorescent powder dyed pupae are packed and banded in polyethylene bags; (i) Irradiated pupae are kept in polystyrene containers with ice packs during the shipment; (j) Irradiated pupae are directly delivered to the emergence facilities by a truck with refrigerated room; (k) Pupae are incubated for emergence in plastic boxes at  $26\text{--}27^\circ\text{C}$  room temperature; and (l) Sterile flies are ground released at the fixed point.

### Quality control

Quality control has been continuously assessed on egg hatch (%), pupal weight (mg), fly emergence (%), flight ability (%), sex ratio and sterility (%). From 2000-2002, quality control of pupae was applied only before and after irradiation. Between 2003-2007 quality control of pupae included other was expanded to other stages such as: 1) before irradiation 2) after irradiation without dye 3) after irradiation with dye and 4) after packing for transportation. Since May 2007 the quality control followed the International Fruit Fly Quality Control Manual (FAO/IAEA-USDA 2003) and involved: 1) before fluorescent dying 2) after fluorescent dying 3) after irradiation and 4) after packing for transportation.

Average quality over 10 years (2000-2009) for *B. dorsalis* and *B. correcta*, respectively, were egg hatch:  $80.01 \pm 5.60\%$  and  $83.78 \pm 2.21\%$ ; pupal recovery:  $37.41 \pm 8.19\%$  and  $21.70 \pm 3.08\%$ ; pupae number per 10 cc:  $463.88 \pm 17.70$  and  $477.17 \pm 7.79$ ; pupal weight:  $11.07 \pm 0.51$  mg and  $11.02 \pm 0.21$  mg. For *B. dorsalis*, the average adult eclosion and percentage of fliers before and after irradiation were  $90.66 \pm 5.01\%$ ,  $83.95 \pm 7.88\%$ ,  $88.85 \pm 5.58\%$  and  $76.95 \pm 7.24\%$ , respectively. For *B. correcta*, the average adult eclosion and percentage fliers before and after irradiation were  $90.09 \pm 3.76\%$ ,  $85.67 \pm 4.52\%$ ,  $87.88 \pm 3.84\%$  and  $81.06 \pm 4.47\%$ , respectively.

In spite of the great advances achieved at the mass rearing facility, there were some drawbacks. The high fluctuation in pupae production was the result of not being able to maintain stable conditions throughout the rearing process, such as air-conditioners broke down, insufficient electric power, lacked of water supply, etc. Inadequate and non-continuously funding was a major constraint that affected the sterile fruit fly support of Thailand. This situation highlights the importance of adequate support to sustain a program successful.

### Surveillance activities

Fixed monitoring sites (Cox and Vreysen 2005) using Steiner traps with methyl eugenol and insecticide bait were the network surveillance traps used throughout the fruit fly control areas. Traps were distributed in a grid-like array with a density of 1 trap/km<sup>2</sup> (IAEA 2003) by using hand-held GPS receivers and GIS technology. All the traps were inspected weekly and trapped flies were examined as sterile or wild flies based on the visualization of dye particles in the sutures and on the *ptilinum* using a microscope with a UV light source. Trapped flies were recorded into GIS to map and visually display the various fixed trapping sites.

Fruit sampling was done twice a month on various types or varieties of fruit in the fruit fly control area, especially mango and long kong (the fruit of most economic relevance) as well as rose-apple, guava, papaya, custard apple, banana, santol, jujube, Marian plum, Carandas plum, Indian cherry, Indian almond, etc. Fruits with signs of infestation were collected and maintained in plastic boxes with sawdust or coir fluff in the bottom to observe flies' emergence. Adult flies were identified and percentage of damaged fruit was assessed for each species of fruit fly.

## Economic assessment

### 1) Ratchaburi

An economic assessment for fruit fly control project in Ratchaburi was conducted (Enkerlin 2001) and included the analysis of four control options: 1) conventional control (low input), 2) conventional control (high input), 3) SIT suppression non area-wide (*status quo*) and 4) SIT suppression area-wide (improved option) (Table 1). For the control options, costs and benefits were estimated for a time horizon of 14 years, which was the amount of time that has passed since the project started in 1988. The total costs and benefits across 14 years were adjusted using a discount rate of 8% based on current inflation and interest rates of Thailand.

**Table 1** Description of the control options analyzed in Ratchaburi province (from Enkerlin 2001).

Option	Description	Damage level (%)
Conventional control (low input)	Minimum orchard management is carried out by farmers. Irregular pest control is done.	50 to 80
Conventional control (high input)	Moderate orchard management is carried out including pruning, orchard sanitation and a conventional pest control programme at orchard level. Oriental fruit fly control includes from 7 to 10 calendar insecticide cover sprays per season.	20 to 30
SIT suppression non area-wide ( <i>status quo</i> )	Moderate orchard management is carried out including pruning, orchard sanitation, minimum level of population monitoring, 1 to 2 insecticide sprays against Oriental fruit fly and permanent ground releases of sterile flies at orchard level.	1.5 to 6
SIT suppression area-wide (improved option)	Intensive orchard management is carried out including pruning, orchard sanitation, optimum level of population monitoring, permanent aerial releases of sterile flies in orchards and marginal areas. A government centralized management structure is in place where farmers actively participate in monitoring and control activities in the orchards and the government takes responsibility for activities in the marginal areas.	<1

The Benefit cost ratio, the net benefits and other related variables are shown in Table 2.

**Table 2.** Economic indices for fruit fly control options analyzed in Ratchaburi province

Option	B/C ratio	Net benefits (US\$ million)	Gross revenues NPV (US\$ million)	Cost NPV (US\$ million)	Pay-back
Conventional control (low input)	1.2	0.12	0.98	0.79	NA
Conventional control (high input)	2.3	1.9	5.9	2.6	NA
SIT suppression non area-wide	7.5	7.5	14.6	1.8	NA
SIT suppression area-wide	10.5	11.3	20.7	1.7	1

*Conventional control (low input):* As the economic figures indicate this was basically a subsistence mango production system where farmers operate at low costs and obtain a very low profit margin. The average farmer in Thailand had around two hectares of mango. Under this control option the total income per year for an average farmer would be of US\$192, which was well below the annual minimum wage in Thailand.

*Conventional control (high input):* In the case of the Oriental fruit fly from 7 to 10 insecticide treatments were done to protect the crop throughout the season. This was an expensive and ineffective control method that did not provide farmers with any comparative market advantage. According to a group of farmers in Thailand mango growers that used this pest control scheme loss 30% of the crop despite the heavy insecticide applications and had no chance to export their fruit because of the insecticide residues and low quality of the fruit. However, the economic indices obtained through the application of this control option were favorable compared to the low input conventional control method described above.

*Non area-wide SIT (status quo):* The total mango area under SIT suppression was 1,120 ha (7,000 Rai) which was only around 0.2% of the total cultivated area in Thailand. Through the application of the SIT for population suppression damage levels have dropped from around 80% before the use of this technology to an average of 4% in the followed seven years (1994-2000) and farmers, by using an environmentally friendly technology, have found better prices for their mangoes in the export market. The economic analysis projected over 14 years indicates a benefit to cost ratio of 7.5 to 1 and a net-benefit of US\$7.5 million.

*Area-wide SIT (improved option):* Due to the more effective use of the sterile flies the cost of an area-wide SIT project would be practically the same compared to the status quo (i.e. non area-wide SIT application). The effective use of SIT would reduce the Oriental fruit fly damage to less than 1% greatly improving the gross revenues compared to the status quo. An additional benefit of the area-wide approach would be the complete elimination of the insecticide use and, apart from the savings from insecticide use, the mangoes could be sold at a higher price through a premium paid by the clients for low insecticide residues and high quality fruit. Since the technology has been used for more than 10 years, it was expected that the major benefits, which were reduction of the current 4-6 % damage (average of the past five years) to less than 1% and savings in insecticide treatments, would be obtained in the first year allowing for the initial investment to be recovered in the same year. The net benefits were positive since the first year and increase gradually as the level of damage was reduced, insecticide sprays were eliminated and mango was sold at a better price in the export market.

## 2) Assessment for an up-scaled SIT program

In addition, an economic assessment for an up-scaled SIT program against the Oriental fruit fly in Thailand was conducted by Knight (2002). Eight different areas were examined for their suitability for area-wide control of the Oriental fruit fly. These included the original project area in Ratchaburi province (Pak Tho) and areas of Petchaburi, Phitsanulok, Chachoengsao, Uthai Thani, Khonkhen, Prachuap Khiri Khan and Phichit province. There was no doubt that Oriental fruit fly can cause significant damage during the main fruit growing season but the levels were relatively low during the off season fruit production. It was difficult to obtain figures on the area of mango that produced off season but the damage levels in this crop was said to be about 5% and can be up to 50% of crop in some areas. Oriental fruit fly damage under different control strategies are given in Table 3 and were used in the analysis.

**Table 3.** Damage levels (%) due to Oriental fruit fly under different control strategies (from Knight, 2002).

Type of fruit		Without control		Chemical control Low input		Chemical control High input		Methyl eugenol		SIT	
		Domestic	Export	Domestic	Export	Domestic	Export	Domestic	Export	Domestic	Export
Mango	In season	100	100	60	5	20	2	5	5	1	1
	Out of season	5	1	2	1	2	1	1	1	1	1
Java apple		100	100	60	5	20	2	5	5	1	1

The area to be treated in each province was calculated from the area of mango that was present within it. The area of the “buffer zone” was calculated in the following way. First, the core area was established and assumed to be a circle. Then, the area of the buffer zone was expressed as a percentage of the core area in an adjacent cell. Most provinces had a buffer area approximately four times the area of the core to represent the dispersed nature of the mango orchards. Two provinces, where mango appeared to be more concentrated, had a smaller buffer zone of approximately 2.7-2.8 times the core area. The cost of SIT would be quite sensitive to the area that had to be treated and it would be a useful exercise to try and identify areas where fruit was particularly concentrated which would reduce the treatment of non-crop areas. Discussions with the geographic information systems analyst within the Department revealed that there were good land use maps that could be used to identify suitable areas.

In order to investigate the impact of having smaller or larger buffer zones this parameter was given a range of values using a triangular distribution and analysed with a Monte Carlo simulation. The actual numbers used varied from province to province but represented a change of about plus or minus 20% of the most likely value.

The release rate of the *B. dorsalis* was set at a standard rate of 500 males per hectare (equivalent to the release of 1000 flies/ha). However, the pilot project in the Pak Tho district used up to 10,000 flies/ha as a release rate. This compensated for the fact that flies were released at a limited number of locations and not evenly over the entire area as was desirable. To investigate the effect of altering the number of flies released per hectare a triangular

distribution was used in the Monte Carlo simulation with a minimum and most likely value of 500 flies/ha and a maximum value of 2000 flies/ha. For the purposes of this analysis it has been assumed that some of the capacity of the production facility was given over to the rearing of *B. correcta* and that these flies were produced at the same cost as for *B. dorsalis*. The *B. dorsalis* were released at the same rate in all locations but *B. correcta* was released at different rates according to the level of infestation of this species. The release rates were done using a triangular distribution and the values used for the different provinces are as in Table 4.

**Table 4.** The release rates of the *B. dorsalis* for the different provinces.

Province	Minimum	Maximum	Most likely
Petchaburi	0	0	0
Prachuap Khiri Khan	0	0	0
Ratchaburi	500	4,000	1,000
Phitsanulok	1,000	4,000	2,000
Chachoengsao	500	4,000	1,000
UthaiThani	500	4,000	1,500
Khonkhen	1,000	4,000	2,000
Phichit	1,000	4,000	2,000

The cost of the flies whilst based on actual figures was felt to be quite low and was therefore included in the Monte Carlo simulation to assess the impact of any changes on the overall profitability of the projects. The variability in the price was expressed as a triangular distribution with a minimum value of \$0.069 per 1000 flies, a most likely value of 0.077 and a maximum value of 0.085 (Cell: Fly Production I52).

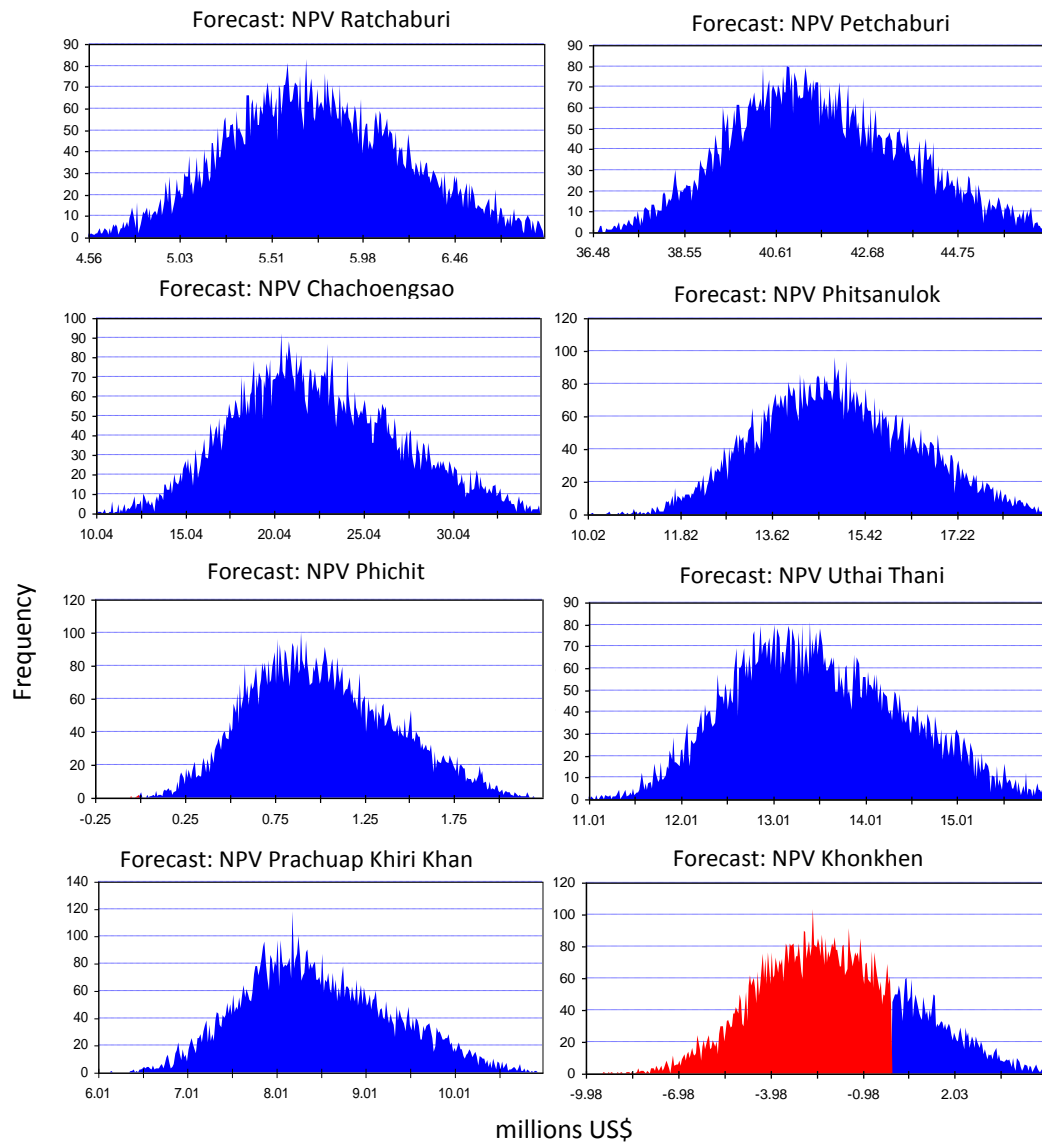
The analysis made no allowance for any changes in the industry such as the expansion of the area of fruit being grown. From discussions with DOAE staff there did not appear to be any national plans to expand the industry and no changes would occur as a result of individuals joining or leaving the industry.

Finally, no allowance had been made for the potential environmental benefits of SIT over conventional chemical control. One reason for this was that chemical control would continue to be used against other insect pests so whilst there would be a reduction in pesticide use it would not be eliminated altogether. Since no figures for pesticide pollution or poisonings were available it was difficult to justify the inclusion of this component. It could be assumed however that some damage was being done and some benefits would come from the use of SIT. More work would have to be done if this figure were to be incorporated.

The final assumption was that the resulting increase in fruit production could be absorbed by the markets and did not result in a decrease in price. Without expensive market studied it was impossible to say whether that was the case. This assumption would tend to overvalue the benefits arising from the Oriental fruit fly control with SIT. Conversely there should be an increase in the production of fruit with lower levels of damage and pesticide residues which could be sold into the more lucrative export market, especially to Japan.

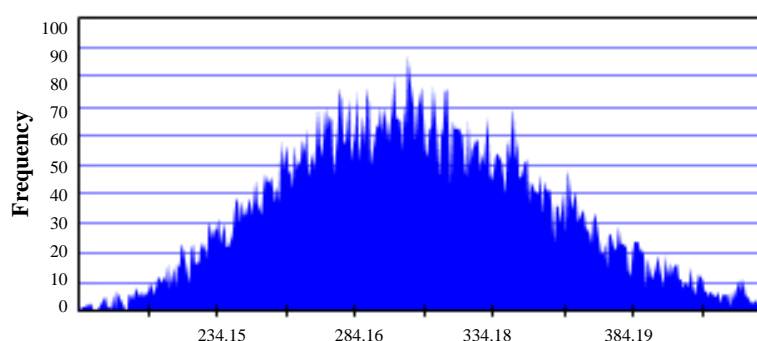


The economic assessment of cost benefit analyses for an up-scaled SIT program against the Oriental fruit fly in Thailand is shown in Fig. 5. The results were the result of 10,000 runs. The figures for NPV were calculated for a period of 14 years, which was a reasonable period of time. The red part of the frequencies indicate that the NPV was less than zero i.e. a loss was being made, the blue part of the curve showed when the projects have a positive NPV.



**Fig. 5.** Economic assessment for an up-scaled SIT program against the Oriental fruit fly in eight provinces of Thailand.

The variation in the number of sterile flies that could be required to successfully suppress the fly population in all of the areas where it was economically desirable to do so is shown in Fig. 6. All areas were included except for Khonkhen.



**Fig. 6.** Forecast. Total number of sterile flies in all areas less Khonkhen.

## Research activities

In the meantime, TINT developed the white thorax strain of *B. dorsalis* in 2007, and extended the research of SIT AW-IPM to other fruit production areas in parallel with consideration of the new sterile fruit fly mass-rearing facilities construction in the near future. Furthermore, the development of the genetic sexing strain was carried out with cooperative of group of people from DOAE and MU under the IAEA Coordinated Research Project.

In order to cost-effectively apply SIT, the possibility to improve young sterile male performance of *B. dorsalis* and *B. correcta* with methyl eugenol and/or adult diet were studied. The extensive series of studies involved more than 2,800 field cage tests with potted mango trees. The effects of different pre-release diets and methyl eugenol (ME) were examined both independently and in combination. Tests were carried out with flies starting from ages of aged 15 and 18 days for *B. dorsalis* and *B. correcta*, respectively. To evaluate the effect of different pre-release diets on males, no-choice mating tests were conducted with sterile males of increasing age and mature sterile females. Sterile males fed up to 2 days of age on sugar-yeast hydrolysate combinations achieved significantly more matings than males fed only water in *B. dorsalis* and more matings than males fed only sugar, only yeast hydrolysate or only water in *B. correcta*. To examine the effect of ME on mating performance, 2, 3, 4 or 5-day-old sterile males were given or not given access to ME for 1 h, followed by the sugar-protein diet until the day of the mating test. Mating performance tests were carried out with ME-exposed and non-exposed sterile males competing with mature wild males for wild females. Results showed a significant mating advantage of ME-exposed over non-exposed sterile males, although at younger ages they were still less competitive than wild males. The interaction of sugar-yeast hydrolysate diet and ME as pre-release treatments for 2 and 3-day-old sterile males was assessed in terms of male sexual competitiveness. Overall, the combination showed an additive effect on increased mating success in *B. dorsalis* sterile males when competing a wild males for wild females, while in *B. correcta* males the drastic improvement in mating success was mainly linked to ME exposure. These results were transferred to the field operation for a more efficient sterile male release.

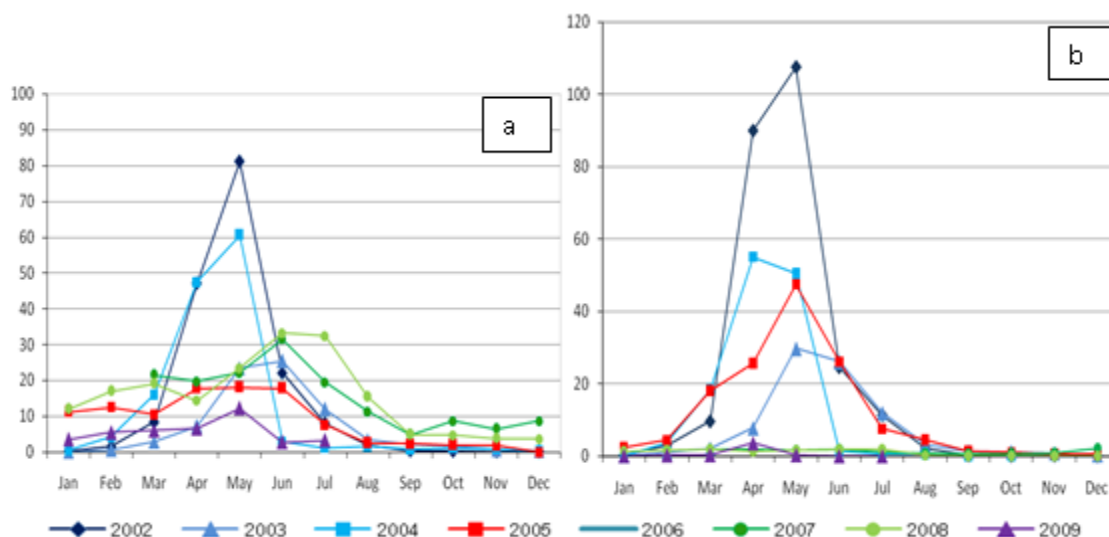
In addition, the research of “Thailand resolution of *Bactrocera dorsalis* complex” which was part of the IAEA’s Coordinated Research Project “Resolution of cryptic species complexes of

Tephritid pests to overcome constraints to SIT application and international trade” contributed to prove that populations from *B. dorsalis* in any geographic region of Thailand is only one species (Chivinijkul et al. 2015).

### Main achievements

From 1987 to 2002, the fruit fly control program of Thailand was focused on *B. dorsalis* exclusively. Since 2003, *B. correcta* was also included, and sterile flies of both species were produced in the mass-rearing facility. The maximum number of irradiated pupae released for both species was 1,311 million in 2007.

Fly per trap per day (FTD) of *B. dorsalis* in Pichit province was reduced year by year during SIT-AWIPM implementation (Fig. 7a) as well as *B. correcta* in the same area (Fig. 7b). Fruit fly populations showed clearly a peak at the end of the mango season. This is according to a huge amount of fallen fruits and ignored harvesting of mango fruits due to low price.

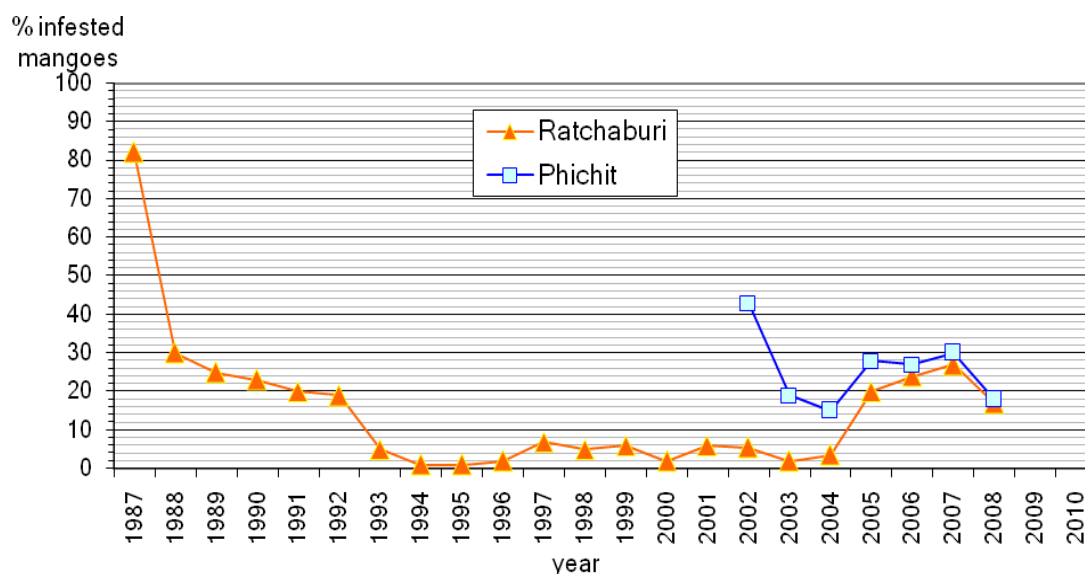


**Fig. 7.** Number of wild flies per trap per day (F/T/D) before the release of sterile flies (2002) and during the SIT-AW-IPM implementation (2003-2009) in Pichit province.

Results from the fruit survey done throughout the release area in which mangoes with signs of infestation were collected and maintained in the plastic boxes to observe the emergence and assessed for the presence of *B. dorsalis* and *B. correcta*, also showed good results.

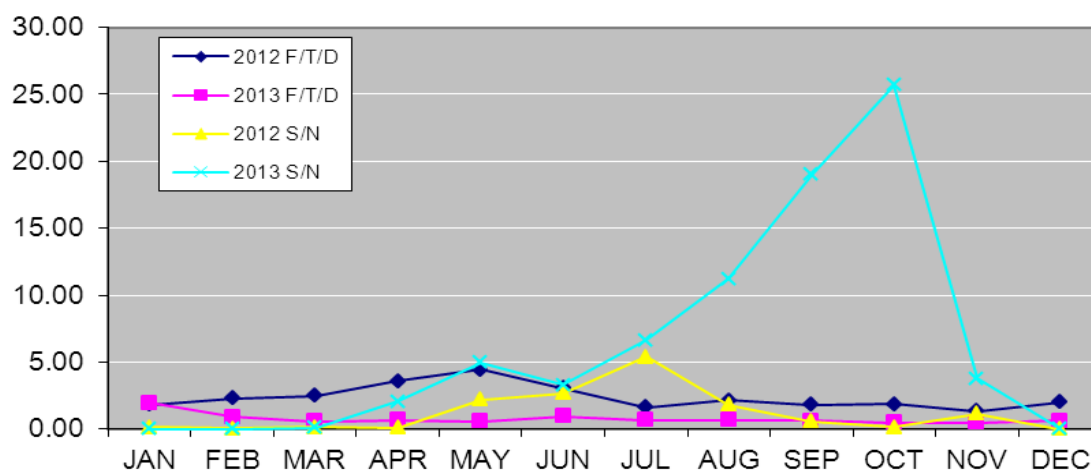
After a decade of non area-wide SIT IPM, at the beginning of the program, percentage of infested fruit in Ratchaburi province was definitely reduced and maintained at low percentage of fruit damaged, that paid good dividends to a small number of mango growers. During 2001-2004, under SIT AW-IPM effort, percentage of infested fruit was maintained at an average of less than 3.6%. Meanwhile in Pichit province where the control program was carried out for 2 years during 2003-2004, the infestation was reduced from 43% in 2002 to 15%. Unfortunately, inadequate budget in 2005 interrupted sterile fly releases and therefore

infestation increased to 17% in Ratchaburi and 23 % in Phichit (Fig. 8) even though growers applied MAT individually in Ratchaburi and through the area in Phichit.



**Fig. 8.** Percentage of infestation in the released areas.

In Trok Nong sub-district, Khlung district, Chanthaburi province, the positive results were shown as a reduction in terms of percentage of fruit damaged, especially longkong, from 30% in 2005 to 5% in 2013. The increased value of longkong and mangosteen were 0.067 and 0.983 US\$ millions, respectively. The average FTD of *B. dorsalis* was maintained at low levels, around 0.67. Conversely, the average sterile to wild ratio (S/N) increased in 2013 compared with 2012 (Fig. 9).



**Fig. 9.** Number of wild *B. dorsalis* per trap per day (F/T/D) and S/N ratio in 2012 compared to 2013.

## Conclusions

In all, the different activities involved in the SIT AW-IPM fruit fly control program in Thailand faced different challenges during more than 10 years of implementation and



achieved many goals. The equipments, methodologies, sterilization process, quality control evaluation and sterile flies release strategy were improved during the past decade since 2002 and reached the standards of the International Fruit Fly Quality Control Manual (FAO/IAEA/USDA, 2003) in May 2007. The integrated SIT approach was effective in controlling fruit flies by reducing damage of fresh fruits. This has opened the exportation possibility of products in the selected pilot areas to some of the most stringent and lucrative markets. High quality mango in Thung Luang sub-district, Pak Tho district, Ratchaburi province and Wang Thap Sai sub-district, Sak Lek district, Phichit province allowed importing greater amounts to more premium market countries. Mangosteen and long kong produced from Trok Nong sub-district, Khlung district, Chanthaburi province achieved higher marketable value than products from other non fruit fly control areas. Important factors that affected the degree of population suppression using SIT were high variability in pupae production, such as inadequate and uncertainty of budget, deficient sanitary practices in the control area, and unacceptable of the released sterile female effect. The economic feasibility study clearly showed that fruit fly control in Thailand using area-wide SIT could be expanded to other production areas with significant economic returns. A national impact should be achieved by scaling-up the project from pilot areas to a country-wide level.

DOAE has expanded AW-IPM without SIT with limited budget and finally without special support for the fruit fly control project in the past few years. The project is still moving forward under either governor offices, local organizations or private sector's vision and subsidy. In addition, the fruit flies control program has been emphasized as a national agricultural strategy and the high executive administrator considers it of importance. It is expected that the planned collaborative project among organizations will be approved and implemented in the near future. DOAE and TINT should scale up the mass-rearing capacity and release the GSS-white-thorax sterile male only strain.

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## Advances in the national programme of fruit flies in Mexico

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### Abstract

The Mexican national program against fruit flies (NPFF), created in 1992, establish under an area-wide integrated pest management (aw-IPM) control measures against *Anastrepha ludens*, *A. obliqua*, *A. striata* and *A. serpentina*, with the aim to create Fruit Flies Pest Free (FF-PFA) and Low Pest Prevalence (ALPP-FF) areas. Among the control measures established, the Sterile Insect Technique (SIT) and the Augmentative Biological Control (ABC) are the key points highlighted in this review.

Here, we present a summary of success of this NPFF since its creation by means of Cost-Benefit studies. To achieve this success, we will also present the improvement of SIT by means of advances in emergence and release facilities, the improvement of a new sexing strain, along improvement of sterile males' mating performance. In addition to the SIT program, the *Anastrepha* spp. mass rearing facilities was also used to mass-produce the braconid parasitoid *Diachasmimorpha longicaudata*, while keeping two other parasitoids, *Coptera haywardi* and *Doryctobracon crawfordi*, under study for its future incorporation into the ABC program.

In conclusion, the NPFF successfully accomplished its purpose, an external advisory board determined a cost/benefit rate of 1/24. Nevertheless, there are still some challenges to achieve a best NPFF in the future, like the development of a dry trap, the search for a parapheromone for *Anastrepha* spp., or to obtain appropriate funding for area-wide with a complex socio-ecological environment.

**Keywords:** area-wide, integrated pest management, sterile insect technique, *Anastrepha* spp., pest-free areas, areas of low pest prevalence.



## Introduction

In 1992, the Mexican Federal Government implemented the National Campaign against Fruit Flies (NCFF), with the objective to control and eradicate the four regulated species of fruit flies considered of economic importance: *Anastrepha ludens* (Loew), *A. obliqua* (Macquart), *A. striata* (Schiner) and *A. serpentine* (Wiedemann). Simultaneously, a trapping system was implemented to avoid the establishment of exotic fruit flies (Reyes *et al.*, 2000).

The technology of eradication used by the NCFF is supported by a system of integrated pest management (IPM) which include monitoring actions (trapping and sampling of fruits) and control (specific bait spraying, cultural activities, the release of natural enemies and sterile flies) (SARH, 1991). The harmonized implementation of these activities are designed to achieve the establishment of free and low prevalence zones of the pest, where the environmental conditions allows it, which will allow the producers to have optimum fruit quality, with the objective that in addition to offer the fruit to the national consumer, also have the opportunity to compete at international markets.

The integrated management of fruit flies activities applied in Mexico, have legal and technical basis on the regulation NOM-023-FITO-1995, which establishes the National Campaign against Fruit Flies (published in the Official Journal of the Federation [DOF] on 11 February 1999). This regulation define the parameters for the recognition of three phytosanitary categories on the basis of pest population indexes, namely: (i) fruit fly free zone; (ii) low prevalence zone; and (iii) under phytosanitary control zones. Lately, and with the objective to strengthen the measures for the protection of fruit fly free and low prevalence zones, on April 23th 1998, it was published the regulation NOM-075-FITO-1997, which restrict (laying down) the requirements and specifications for host fruits shipment.

The present work shows how the Mexican producers had deal with the increased control measures, and how the country had established each phytosanitary zone that allows an international trade of different commodities.

## Materials and Methods

### *Regulations, collaborative agreements and joint programmes*

Strategic alliances among the Federal Government, State Governments and producers, were established, to combine efforts and economic resources to implement the Strategic Plan for the control of the pest. A Cooperation Agreement with the Interamerican Institute for Cooperation on Agriculture (IICA), for the production of sterile flies *A. ludens* and *A. obliqua*, and the parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), was signed. In the same way the Institute of Ecology AC, and the International Atomic of Energy Agency (IAEA) had collaborated implementing specific projects to perform basic and applied research. Official documents had been published as standards, manuals procedures as legal and technical work frame to support the operations of the NCFF.

To encourage the participation of fruit producers in the NCFF, the Federal Government, State Governments, and the industry sign annual collaboration agreements to provide resources for the operation of the NCFF. The activities of the NCFF are performed by state plant health committees made up by growers. The activities of those committees are supervised by official staff and validated by verification people.

#### *Mass rearing facilities and surveillance methods*

To implement strategies for autocidal control (SIT) and biological control (parasitoids), the NCFF has a facility, the Moscafrut facility, for mass rearing of sterile fruit flies (*A. ludens* and *A. obliqua*) and the parasitoid *Diachasmimorpha longicaudata*. The Moscafrut facility is located in Metapa de Dominguez (Chiapas), and produces a weekly average amount of 125, 60, and 25 million of *A. ludens*, *A. obliqua* and *D. longicaudata*, respectively (Gutiérrez & Santiago, 2008). The sterile *Anastrepha* males and parasitoids are sent to different States where fruit fly suppression programs are carried out by their release within IPM program (Table 1).

Sterile fruit flies are packed using “Mexico” type towers and after emergence (Hernandez et al., 2010), the adult flies are supplied with juvenile hormone (through the food), in order to accelerate sexual maturity (Gómez et al., 2013). On 2014, the SIT was applied on 100 thousand hectares of citrus, mango and guava through the operation of five centres for Packing and Release sterile flies under the system of chilled adult and aerial releases.

**Table 1.** Production and distribution of sterile flies and parasitoids during 2014.

State	Pupae of <i>A. ludens</i>		<i>A. ludens</i> strain Tap 7.		Pupae of <i>A. obliqua</i>		Puparia of <i>D. longicaudata</i>	
	Millions	%	Millions	%	Millions	%	Millions	%
Special shipments	3.99	0.07	5.36	1.14	6.48	0.21	0.19	0.02
Guerrero	0.00	0.00	0.00	0.00	0.00	0.00	203.08	19.27
Nayarit	624.72	10.22	0.00	0.00	662.13	21.63	0.00	0.00
Nuevo León	752.81	12.31	0.00	0.00	0.00	0.00	0.00	0.00
Chiapas	517.93	8.47	430.62	91.30	489.86	16.00	284.15	26.96
Oaxaca	0.00	0.00	0.00	0.00	0.00	0.00	48.20	4.57
San Luis Potosí	2854.96	46.69	0.00	0.00	0.00	0.00	0.00	0.00
Sinaloa	546.51	8.94	0.00	0.00	1832.36	59.86	154.64	14.67
Zacatecas	751.50	12.29	0.00	0.00	0.00	0.00	240.12	22.78
<b>Total Material Sent</b>	<b>6052.42</b>	<b>98.99</b>	<b>435.98</b>	<b>92.43</b>	<b>2990.83</b>	<b>97.70</b>	<b>930.38</b>	<b>88.27</b>
Colony	61.89	1.01	33.97	7.20	70.34	2.30	121.99	11.57
Cull	0.00	0.00	1.73	0.37	0.00	0.00	1.69	0.16
<b>Total of pupa produced</b>	<b>6114.31</b>	<b>100.00</b>	<b>471.68</b>	<b>100.00</b>	<b>3061.17</b>	<b>100.00</b>	<b>1054.06</b>	<b>100.00</b>

In 2014, to protect the free zones throughout the year there were operated 8,109 McPhail or Multilure traps, baited with hydrolyzed protein or torula yeast. When a fruit fly or more are found, immediately an emergency plan is activated to eradicate the detection or outbreak of fruit flies.

In the low prevalence zones in order to strengthen the eradication process, during 2014 every week 9,548 traps were checked; there were sampled 184 ton of fruit; destroyed 2,632 tons of fruit as mechanical control; 164,877 hectares were sprayed with specific selective bait; about

9,479 million sterile flies (*A. ludens* and *A. obliqua*) were sent, mainly to, the states of Chiapas, Nayarit, Nuevo Leon, San Luis Potosi, Sinaloa and Zacatecas. Additionally, 930 million of parasitoids were sent, mainly to, the states of Chiapas, Guerrero, Oaxaca, Sinaloa and Zacatecas.

In addition, for the protection of the fruit fly free and low prevalence zones, there are 29 internal verification road-stations. Annually about 556 ton of host fruit are seized; 335 ton of fruit are sampled; 74 tons of fruit fly hosts commodities are fumigated and 6.2 million vehicles entering or passing through fruit fly free and low prevalence zones are inspected

On the zones under plant protection during 2014, activities included: monitoring through a trapping system made up by 7,753 traps, fruit sampling, mechanical and chemical control. The results of these measures yielded in: reduction of production losses caused by fruit flies, reduction of pesticides use and the expansion of market opportunities for national fruit commodities.

## Results and Discussion

### *Establishment of phytosanitary zones*

From 1992 to 2014, Mexico has declared the following zones as fruit fly free zones: the states of Baja California, Baja California Sur, Sonora, Chihuahua, Coahuila, 36 municipalities in the State of Zacatecas, 32 municipalities in the state of Durango, 27 municipalities in the state of San Luis Potosi, 24 municipalities in the state of Nuevo León, 12 municipalities in the state of Sinaloa, one municipality in the state of Tlaxcala and some communities in the state of Morelos. Similarly, zones of low prevalence were established in municipalities of the states of Tamaulipas, Nuevo León, Zacatecas, San Luis Potosí, Guerrero, Durango, Nayarit, Sinaloa, Michoacan, Aguascalientes, Puebla and some communities of the states of Estado de México and Morelos.

The NCFF has very important achievements: Mexico has 51.10% of its territory, equivalent to 1,000,242.57 km<sup>2</sup> of fruit flies free zones, and within these zones there are about 85,000 hectares of fruit crops (citrus, mango, apple and peach). Whereas only 9.75% of Mexico's territory has a phytosanitary status of low prevalence for *Anastrepha* fruit flies, equivalent to 190,932.97 km<sup>2</sup>, within these zones, there are 186,000 hectares of orchards such as citrus, mango, guava and peach (Fig. 1). On 2014 it has been possible to export without a post-harvest quarantine treatment 36,518.5 tons of mangos, 20,482 tons of orange and 764 tons of peach, with an annual market value of over 50 million US dollars.



**Fig. 1.** Distribution of phytosanitary zones in Mexico, 2014, according to phytosanitary zone classification depending on prevalence of Tephritid fruit flies (*Anastrepha ludens*, *A. obliqua*, *A. striata* and *A. serpentina*). In green the fruit fly free zones, in yellow the low fruit fly prevalence zones, and in red the zones under phytosanitary control.

In addition to this data provided by own NCFF, different countries had recognized 202,017 km<sup>2</sup> of fruit fly free zones in Mexico such as USA, Australia, New Zealand, the European Union and Japan (Gutiérrez & Santiago, 2008).

#### *Mass rearing facilities and surveillance methods*

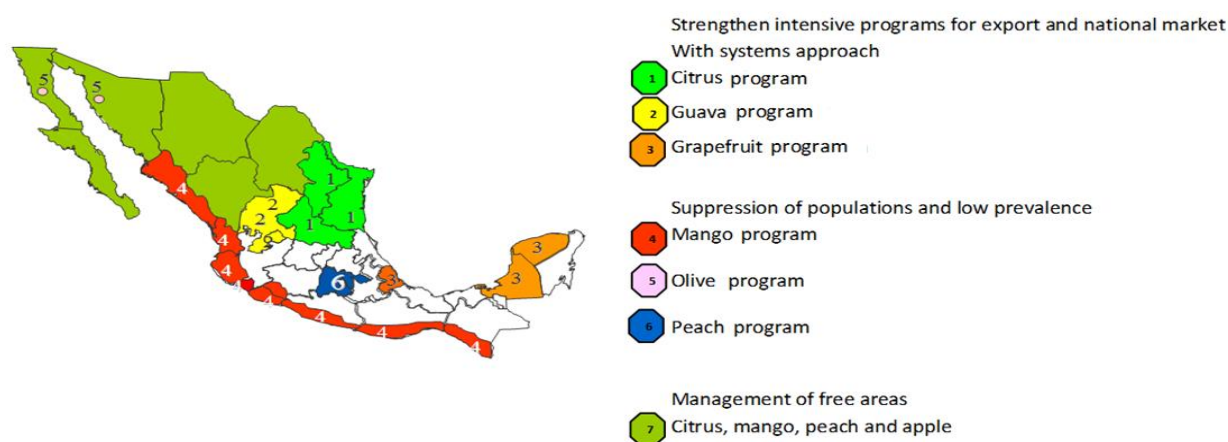
At the Metapa mass-rearing facility, the Methods Development department have strains of two additional hymenopteran parasitoids, *Coptera haywardi* (Ogloblin) and *Doryctobracon crawfordi* (Viereck), which in short time could be scaled to mass production with the aim to strengthen the biological control of the pest. These two larval-pupal parasitoids were described as native of New World, being adapted to the climatic conditions, which would prove the success of mass-rearing and release (López et al., 1999; Aluja et al., 2009).

In the project of genetic sexing, a new strain of *A. ludens* which produces black pupae females, and a brown pupae male was obtained (Zepeda-Cisneros et al., 2014). This allows the selection of only males to be released in the field. This condition is a relevant advantage for the SIT, so the evaluation of the quality of the strain, and scaling it for mass production has been started.

#### *Preventive measures and fruit fly invasions*

Currently, in coordination with the producers and state authorities, regional programs have been developed within each state. The purpose is to redirect the human and financial resources to get an improvement of the phytosanitary status and move toward other areas to

cover the entire State. For this purpose the following plant health programs: mango, citrus fruits, guava, peach and olive (Fig. 2), were prioritized.



**Fig. 2.** Prioritization of phytosanitary programs for fruit fly control.

For each commodity, orchard certification and risk mitigation measures were established, especially for those commodities devoted to international export trade to countries with quarantine protocols. As an example, the trade of mangoes and citrus to the United States is subjected to orchard certification, pre-harvest field operations (which include verification of crop field status, sanitation process, fruit fly monitorization, sterile males releases, ...) and post-harvest treatments (in the case of mangoes, this implies the hydrothermal treatment to kill any fruit fly immature stage) prior obtaining a trade passport.

Since 2007, the procedure for mango orchard registration orchards and its phytosanitary monitoring is performed through SIGMOD, an online system for managing documents and field operations, which allows to trace the fruit origin, treatments received and final market. This new system protect certified fruits while reduce waiting times for producers and packing facilities, and facilitate Federal Government determination of global production.

During 2014, 223,521 tons of mangos were exported to the United States, using hydrothermal treatment. The exporting states were: Michoacan (61,688 ton), Sinaloa (55,720 ton), Nayarit (38,443 ton), Oaxaca (32,454 ton), Chiapas (26,927 ton), Jalisco (7,632 ton) and Guerrero (655 ton).

In addition to the fruit fly-free zones described for 1992-2014 period, during 2014, orchards were certified as temporarily free of fruit flies in the states of Aguascalientes, Campeche, Chiapas, Guanajuato, Jalisco, Michoacan, Morelos, Nayarit, Tamaulipas, Veracruz, Yucatan and Zacatecas. In total 1,826 hectares were certified, distributed as 703 ha of mango, 342 ha of guava, 277 ha of grapefruit, 117 ha of pomegranate, 109 ha of orange, 77 ha of guanabana, 55 ha of plum, 47 ha of peach, 42 ha of tangerine, 28 ha of fig and 26 ha of mamey (SENASICA, 2014).



The risk of introduction and establishment of exotic fruit flies to Mexico is increasing, due to the following factors: 1) increase and diversification in the trade of horticultural products; 2) facilities that provide new means of transport; 3) the increase of tourism; and 4) increase in the number of immigrants arriving in the country to work or that are in transit toward the USA and Canada. To face this risk, as part of the Fruit Flies National Program, Mexico's Federal Government operates the Preventative trapping system against exotic fruit flies (PTSFF) throughout the national territory, to early detect any introduction of Mediterranean fruit fly (*Ceratitis capitata*), Oriental fruit fly (*Bactrocera dorsalis*) and melon fruit fly (*Bactrocera cucurbitae*), among other species of exotic fruit flies. This preventative trapping system protects 1.7 million hectares of crops, with an estimated annual value of 4,500 million US dollars. The system has been in operation since 1996, to mitigate risks of introduction, colonization, establishment and spread of exotic fruit flies. In total 28,255 traps of the PTSFF are reviewed every 14 days, including the detection system of the Regional Program MOSCAMED in Chiapas State, where in some cases also are reviewed every 7 days (SENASICA, 2014). The reports and results of the PTSFF are managed on the "MEXOFRUT" online system at <http://moscascelafruta.sinavef.gob.mx/mexofrut/login.php>. In 2014, there were 726,534 revisions of traps, with national average of 97.7 % of revised traps (SENASICA, 2014). Based on the results of PTSFF during the last 17 years, it can be stated that Mexico is free of exotic fruit flies, serving also as natural barrier to north limiting countries.

## Conclusions

The establishment of a fruit fly IPM program under the area- wide concept, where sterile insect technique is included, is fundamental to achieve the recognition of fruit fly free and low prevalence zones, which allow the export of fruit without further quarantine restrictions.

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## Suppression of Mediterranean fruit fly using the Sterile Insect Technique in Neretva River Valley of Croatia

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### Abstract

**Background:** The Mediterranean fruit fly, *Ceratitis capitata*, (Wiedemann), is a pest of high economic importance in Croatia attacking several cultivated (*Prunus armeniaca*, *Prunus persica*, *Prunus domestica*, *Ficus carica*, *Malus domestica*, *Pyrus communis*, *Citrus reticulata*, *Citrus sinensis* and *Diospyros kaki*), wild (*Arbutus unedo* and *F. carica*) and ornamental host plants (*Eriobotrya japonica*, *Fortunella kumquat*, *Feijoa sellowiana* and *Citrus aurantium*). In Neretva Valley, *C. capitata* affects production of mandarins, *C. reticulata* mainly for export to the EU and Russia, with annual yield valued at over 25 million euros. Beside infestation in mandarin fruits, medfly also cause problems to exports due to quarantine and food safety regulations. After conducting economic and technical feasibility studies, two years of successful suppression through a Sterile Insect Technique (SIT) based IPM pilot project, the Croatian Ministry of Agriculture expanded the project to the lower part of the Neretva Valley, covering over 4000 hectares of fruit orchards, mainly mandarins. Since 2013, growers' associations from Neretva Valley representing producers and industry joined the project funding in terms of supporting part of the labour, energy and sterile males costs.

**Methods:** Fly emergence and release facility was build and equipped in the city of Opuzen, with current packing capacity of up to 30 million sterile flies per week. Releases of sterile males are performed mainly with two ground release machines mounted on vehicles, using chilled flies. Trapping system is set and geo-referenced over the whole SIT treated (4000 ha) and non-treated area of the valley (additional 4000 ha) with Tephri Traps using 3 component lures (ammonium acetate, trimethylamine and putrescine) as attractant. Captured flies are checked using fluorescent lamps to separate marked sterile released from the wild flies and to provide information on the insect population levels. Routine fruit sampling is undertaken to evaluate fruit infestation and the efficacy of the suppression methods.

**Results:** Results after two years of the application of SIT at a pilot scale and after two years of expanding the treated areas in a semi area-wide approach showed that medfly population, measured as the number of larvae per kg of fruit, was significantly reduced. In comparison to non-treated area in the upper part of the valley, medfly population was reduced by 92.4% in figs, 73.9% in peaches and 96.8% in mandarins during 2012 and by 100% in figs, 57.3% in peach and 96.7% in mandarins during 2013.

**Conclusions:** The use of SIT to suppress medfly populations in the Neretva Valley proved to be effective providing greater crop protection to the commercially available crops with no negative environmental impact. Future efforts will be aimed at expanding the project to all fruit production areas of the valley in a full area-wide approach switching to aerial releases of sterile flies to target the whole population of the pest improving the effectiveness of the sterile insects.

**Keywords:** *Ceratitidis capitata*, fruit exports, ground release, mandarins, sterile insect technique.

## Introduction

The Mediterranean fruit fly, *Ceratitidis capitata*, (Wiedemann) (Diptera, Tephritidae) is a pest of great economic importance in the Neretva Valley, affecting production of several important crops. Its negative effects are manifested mainly in the export oriented production of mandarins *Citrus reticulata*, Blanco. The pest was first detected in the Neretva Valley in 2001 (Pelicarić et.al., 2001; Pelicarić & Bjeliš, 2002; Bjeliš & Pelicarić, 2004). It was estimated that the damage this pest causes to the mandarin production in orchards as well as the economic damage observed in the export activities reach a total of 10 to 30% depending mainly on how climate affects the pest density (Bjeliš et.al., 2007).

The Neretva valley extends to 15,000 hectares of Croatian territory, of which 7,000 hectares are agricultural areas, where mandarins are the most economically important commodity. Mandarin production in Croatia supplies the needs of the domestic market and 75% of total annual yield is exported mainly to countries of former Yugoslavia, the EU and the Russian Federation. Total annual production of mandarins in the Neretva Valley, varies between 40,000 to 60,000 tons with an increase trend due to a considerable acreage of young plantations coming into production. In the past five years, the average estimated annual production has increased to 100,000 tons.

In order to organize and implement legal measures for the suppression of *C. capitata*, various analysis were conducted to define and adopt the most efficient control (Bjeliš, 2004). Prospective economic and tehcnical feasibility studies showed that the Sterile Insect Technique (SIT) applied as part of an integrated pest management approach, was the best method for suppression of the pest in the Neretva Valley. The economic analysis resulted in a 1 to 6 cost-benefit ratio, meaning that one invested Euro produces a return of 6 Euros or 600% return on investment (Bjeliš, 2007). Also, Neretva Valley is one of the most northern citrus production areas in Europe, being located at the limits of the northern distribution of *C. capitata*, with low winter temperatures affecting its survivorship and the lenght of the life-cycle. In order to understand the full benefits of the application of the SIT, it is important to mention the presence of several bird and fish sanctuaries that are natural reserves and protected sites in the surrounding area of the Neretva Valley. This favors the usage of environment friendly pest control methods. Taking into consideration that *C. capitata* infests ripen fruits, farmers applied broad spectrum insecticides during that period of fruit development. The EU market is

very rigorous and sensitive to the presence of pesticides residues. Moreover, some importing countries such as Serbia or Russia (which represents a significant and constantly growing export market), consider this pest on the A1 quarantine pest list, which include a very demanding set of phytosanitary regulations for mandarin exports from the Neretva Valley (Bjeliš et al., 2008; Bjeliš et al., 2010).

Due to the reasons presented above, the agricultural authorities of Croatia decided to apply SIT, a widely proven environment friendly control method for pest suppression. SIT is a highly selective method that involves laboratory mass rearing and sterilization of a large number of medfly males, which are then ground released into the commercial orchards and surrounding marginal areas (Hendrichs et al., 2002.). The sterile males are released twice a week at 1500 flying males per hectare, where they compete with wild males for matings with wild females (FAO, 2007). The continuous matings between sterile males and wild females result in the reduction of the offspring and less larvae in the fruits.

In order to properly carry out the implementation of the SIT method, the Ministry of Agriculture, Fisheries and Rural Development of Croatia has provided capital investment for the construction and equipment of the sterile fly emergence and release facility for the implementation of the SIT programme with the important collaboration of the International Atomic Energy Agency (IAEA) through its Technical Cooperation Programme.

## **Material and Methods**

### *Construction and equipping a sterile fly emergence and release facility*

A facility for the implementation of the SIT programme was built in 2010. It is located in the city of Opuzen in Neretva river valley (Fig.1), Dubrovnik - Neretva county and it occupies an area of 180 m<sup>2</sup>. The facility, in its full capacity, enables the development of 30 million sterile pupae per week, which meets the needs of the program for the entire fruit growing area of the valley.

The funds are not yet sufficient to treat the whole area, so the program now operates and covers a total of 4000 ha, where the majority of the mandarin plantations are located.

The facility is organized into several rooms with the purpose of ensuring an adequate management of the sterile flies and consists of:

1. Reception and packing room for sterile medfly pupae: controlled laboratory conditions, equipped with volumetric automatic packing line and paper bag sealing machine
2. Laboratory for quality control of the sterile pupae: controlled laboratory conditions, equipped with pupal counter machine, precision balance, plexiglass cages for adult holding, aspirators for sterile males handling, freezer etc.
3. Food preparation room: equipped with a device for mixing the finished adult food, balance, equipment for agar food preparation, boiling machine etc.



4. Two separate holding rooms, approximately 30 m<sup>2</sup> each for the emergence and development of sterile males: controlled laboratory conditions, humidifiers and dehumidifiers, day – night conditions, air changing equipment, trays for paper bags, 25 towers type Mubarqui
5. Chilling room for the cold treatment and collection of the flies prior to its release with ground release machines in the field: chilling device, collection table
6. Dark room: laboratory for the discrimination of sterile and wild flies and identification of the fruit flies captured in traps: UV lamps, fluorescent binocular
7. Fruit sampling room for the development of collected fruits of Medfly hosts and examination of samples of fruit of export shipments: controlled laboratory conditions, drying device, 3 tables for collection of larvae from Infested fruits sampled, pots etc.
8. Office with computers used for data entry and the archive of reports and collected data.

Apart from the above mentioned infrastructure, for the outdoor field operations the programme uses four vehicles of which 3 are pick-up models, 3 ground release machines for the release of chilled sterile flies and 2 boats to cover areas only accesible by water channels.



**Fig. 1.** Neretva river valley – main mandarins cultivation area in Croatia.

#### *SIT treated area from 2010 to 2013*

The suppression of the Mediterranean fruit fly began as a pilot project in 2010, with the construction of the facility for the handling of sterile flies. The treated area covered only a semi-isolated mandarin production area of about 1000 ha (Bjeliš et.al., 2010; Bjeliš et.al., 2013). This area is located in the southern part of the valley, between river Mala Neretva to

the north, bordered by a mountain chain to the south and east and the sea to the west. In order to prevent invasion from the surrounding areas, certain control measures were carried out in 10 villages that were defined as buffer areas. During the year 2011 the treated area expanded to an additional 50 ha of city of Opuzen with settlements along the coast of river Mala Neretva and 200 ha of plantations near Opuzen for a total area of 1250 hectares to be treated with sterile insects. During 2012, the area under treatment further increased to the northern border of the valley covering 4000 ha where the majority of mandarin and other commercial fruit plantations in the Neretva Valley are located. This same area was treated in 2013.

#### *Pre-release operations*

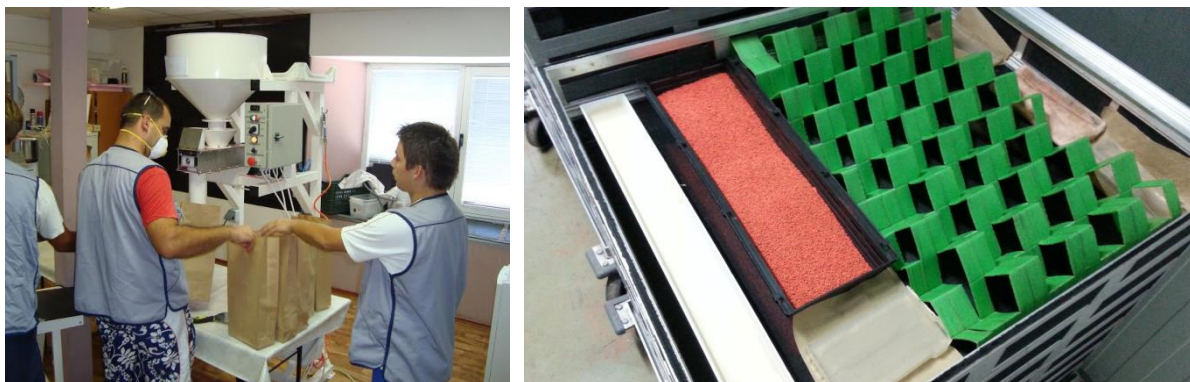
Pupae of sterile medfly males Vienna-8 arrived in the facility in Opuzen in the amount up to 14 million per week in two separate shipments during the period from mid April to the end of November. During 2013/14, 350 million sterile males, were packed, retained and release in the field. Sterile pupae were supplied from the rearing facilities in Israel and Spain. Shipment of pupae in transit must meet certain requirements to prevent damage. The box which contains the pupae packed into small plastic bags has to be made of firm material in order not to get damaged during handling at airports. Also, optimal temperature must be maintained inside the boxes so they are often made of polystyrene. The boxes should be clearly labeled indicating that “it contains living organisms, handled carefully“, as well as the recommended storage temperature during transportation, which must be within 15-20 °C. To achieve this temperature, it is necessary to include artificial ice and a data logger–USB stick inside the consignment in order to measure and store information on temperatures during shipment (Bjeliš et.al., 2013).

Two packaging and holding methods for two different sterile fly release methods are used. For the sterile fly releases at locations and terrains that are hardly accesssible to vehicles, flies are packed and released from paper bags, where bags contain from 3000 to 5000 pupae each.

For the release over fruit commercial orchards and urban areas with good accesibility, the Mubarqui towers with packing and holding system is used. These towers are actually cages for emergence and holding of sterile flies. Mubarqui towers consist of 16 levels each with a maximum capacity of 50,000 pupae per level or 800,000 pupae per tower. Before packing, sterile pupae are placed into a pupal counting machine in order to measure the volume of flies for volumetric packing. These data are relevant for the next phase, which is dosing the pupae using a semi-automated volumetric filling machine with a pre-made range of volumetric units in order to select the one that responds to the given volumetric value (Fig.2). The food used in paper bags is dry adult food, Mubarqui type consisting of sugar and proteins, but with no water so it is mandatory to add water in the holding room after flies start to emerge inside the bags. Water can be taken by the flies through the paper permeable material. For the Mubarqui tower system, dry food based on sugar and proteins is used as well, but with addition of agar cakes that contains both food and water and also pillows soaked with additional water. Each tower level is equipped with long rectangular plastic strips in zig-zag

shape to increase resting surface for adults and ensure adequate space for the sterile males preventing its damage (Fig.2).

After the packing, paper bags and towers containing sterile pupae are placed into holding rooms, in climatized and controlled conditions that ensured 21 to 25 °C and 55- 65% relative humidity (Fig.3). A mechanism for humidification that controls the relative humidity in the air, a dehumidification device that annulates external influences by collecting and accumulating extra humidity and “day-night“ lighting system that simulates natural conditions of daylight duration. A ventilation system is also built-in which enables the exchange of the complete air volume in the rooms 1-6 times per hour in order to eliminate CO<sub>2</sub> produced during sterile flies maturation. A day or two after packing, flies begin to emerge from pupae and stay for maturation in controlled conditions of holding rooms.. The fruit flies are held in holding rooms approximately for 6-7 days, after which are released in nature. In order to improve the sexual performance of the sterile males and encourage their sexual activity in the fields, 24 hours before the release they are exposed to aromatherapy ventilated air with 0,3 ml/m<sup>2</sup> of ginger root oil (Shelly et al., 2007).



**Fig. 2.** Automated volumetric filling machine (left) and Mubarqui towers with accessories and sterile medfly pupae in pupal container (right).

During the process of sterile flies reception, packing, maturation and after chilling, quality control tests are conducted on a weekly basis (Calkins & Parker, 2005). After sterile pupae reception in the facility, the boxes are open and temperature of the packed pupae is checked. After weighing of each bag containing the pupae and checking indicators of sterility, the status of hypoxia in the bags with pupae is also checked. An average sample is taken to the laboratory to run the routine standard quality control tests (FAO/IAEA/USDA, 2014). The routine QC tests carried out in Opuzen facility are: pupal weight, sex ratio, emergence after 48 and 72 hours after packing and at the day of release, flight ability tests in black tubes and stress test. Tests to measure the impact of the cold treatment based on the percentage of flyers after chilling is also carried out. Furthermore, the total amount of collected flies is measured in kilos and the average weight of one adult fly in order to calculate the release rate. In

addition to these tests, field cage tests are run in order to check matting competitiveness of sterile males compared to the wild flies (Bjeliš et.al. 2013).



**Fig. 3.** One of two holding rooms for sterile flies maturation (left) and collection of sterile medfly males in chilling room (right).

### *Release operations*

Release of sterile medfly males in Neretva river valley is conducted as ground release of adults as it used in the other programmes of similar size of the treated area (Dominiak et al., 2003; Barnes et al., 2004). The release of sterile males from paper bags is used on small parcels intersected with numerous water channels and terrains not accessible to vehicles. Release of paper bags covers 45% of the entire treated area using in average 1,300 paper bags with 3 to 5 million of sterile flies per week. The release in the field is conducted according to 10 predefined release routes. Paper bags are opened in each release point by walking in the lines inside the fields in order to get good coverage and flies dispersal with the goal to obtain an average density of 1,500 flying flies per hectare. Important areas of the valley accessible only through the water channels are treated using two boats releasing the sterile flies along the channels where the fruit orchards are present.

The sterile males that are packed in the Mubarqui towers are released using the ground release machines mounted on pick-up vehicles (Salvato et al., 2003). Once the flies are ready for release, they are knocked down by temperature in chilling rooms, collected and transferred to the ground release machines. The cold treatment is conducted in a chilling room equipped with a cooling system which allows the room to reach in a given time the required 1°C (Fig.3). With this temperature flies are shocked which makes them immobile. Once the flies are chilled, all accessories from each level of the Mubarqui towers (the food containers, pads and plastic strips) are taken out and flies are placed in collection tables. Up to 6 million sterile fly adults are being collected in this manner on a weekly basis. Chilled adult flies are then transferred to the release machines, which are also precooled at 2-3 °C.



The program is using 3 newly adopted ground release machines with a capacity of up to 3 million sterile child flies each (Bjeliš & Popović, 2012), that were improved from the first ground release machine prototype built in 2011. (Bjeliš et al., 2013).

The machines are fixed on iron structures so they can be placed and displaced from the pick-up vehicles as needed (Fig.4). The releasing machine contains a cooler system with an aluminum container where a temperature of 2 to 5 °C is maintained during the release time allowing proper handling of the flies during release. The system is driven by two sources of energy: gasoline compressor engine that runs the cooler and the blower while the screen auger rotation and ventilation are connected and work on 12 V power from the car battery of the vehicle. The driver manages all operations from the vehicle by using a control box to switch on or off the ventilator and also to define the rotation speed of the screw auger that regulates the speed and quantity of sterile flies that can be released considering the car speed. The releases in the field are carried out based on pre-determined routes assessed by GPS system (Fig.4). By this release method approximately 55% of the total area is treated.



**Fig. 4.** Ground release machines mounted on vehicles (left) and releasing of chilled sterile males by ground release machine (right).

### *Trapping system*

Trapping system for medfly population survey in area-wide programmes involving the use of the Sterile Insect Technique has been developed in order to support and harmonize trapping protocol in fruit fly control programmes (IAEA, 2003). In order to monitor the occurrence and captures of the Mediterranean fruit fly, and to discover new hot spots throughout the Neretva Valley, a trapping network is set of approximately 140 Tephri traps, in the treated and the untreated area (Bjeliš et al. 2013). All traps use the 3 component lures: trimethylamine, ammonium acetate and putrescine with the addition of a DDVP insecticide chip. Beside the use of this trapping system for the detection of outbreaks and wild medfly population fluctuation during the season, it is also used to compare the ratio of sterile and wild males in the field. All traps are being georeferenced, identified and controlled on a weekly basis. The



content of the traps are transferred to the laboratory for sterile:fertile discrimination under the fluorescent black light. Since the sterile pupae are treated with fluorescent powder before shipping, when examined in the laboratory for identification under UV lamps, sterile males are clearly distinguished from the wild ones (Enkerlin et al., 1996). With this method, the dispersion, spatial distribution and relative density of the sterile flies in the treated area is weekly evaluated. The trapping data are presented as percentage of traps with sterile flies (distribution), flies per trap per day (FTD) and the ratio between sterile males and wild males is compared.

### *Fruit infestation*

In order to determine and evaluate the effect of the SIT method and calculate the efficiency of reduction of damage by medfly, primary medfly host fruits are being sampled. Maps with host orchards distribution are developed with information on the precise location of parcels of the primary medfly hosts in the valley such as mandarins, *Citrus reticulata* B, apricots, *Prunus armeniaca* L., plum, *Prunus domestica* L., peach and nectarine, *Prunus persica* L., fig, *Ficus carica* L., persimon, *Diospyros kaki* T. etc. with their maturation time. Collected fruits are transferred to the fruit sampling room, counted, weight and placed on small containers or tables for collection of larvae with mesh in order to collect all medfly larvae (Fig.5). Larvae collection is recorded every day during 3 weeks after placing the fruit in the holding containers. With this aim, over 2 tons of different medfly host fruits were collected every season during the maturation period to evaluate the infestation and calculate effectiveness of SIT. Fruit infestation is expressed as number of larvae per kilogram of fruits and number of larvae per fruit. With such data, it is possible to calculate the effectiveness of the suppression method between the treated and infested area using the formula developed by Abbott (Abbott, 1925). This formula is recommended when data on infestation or live individuals is available for uniform populations.

*Efficacy by Abbot (%) =  $100 \times (mc - mt) / mt$* , where *mc* is mean number of live insects in treated and *mt* is mean number of live insects in no treated (Abbott, 1925).

### *Sanitation*

Sterile Insect Technique (SIT) is the most efficient when applied as a tactic in the system of the area-wide basis together other technique that will target the total population of the pest over a period of all generations through the season (Klassen, 2005). In order to detect the first infestation produced by overwintering generation of medfly, large numbers of small fruit samples are collected every week following host fruits maturation, starting with apricots, *P. armeniaca* and peaches, *P. persica*. These samples are brought to the laboratory and inspected in order to find medfly larvae. When larvae's are detected in specific orchard or in several orchards of the same crop, a systematic sanitation process is undertaken on a weekly basis and all fallen fruits and fruits remaining on the trees are collected and destroyed (Fig.5). The removal and destruction of the infested fruits is only way to destroy immature stages of pests that cannot be controlled by other methods. Sanitation as a cultural control can significantly

reduce medfly population when it is applied early in the season when the population level is low and immediately after detection of outbreak (Mangan, 2005). Sanitation is focused on the stone fruits, mainly apricots, *P. armeniaca* and peaches, *P. persica* and is organized in a way that all stone fruit orchards are mapped and sanitation is carried out from the beginning to the end of harvest.

#### *Control of export shipments*

In addition to the above procedures, the rate of the presence of the medfly larvae in the export shipments of mandarin is carried out. Approximately 40 samples per season are taken from the export shipments. The sample consists of 200 fruits that are individually placed on holding cages for maturation and the infestation is monitored and assessed. All these operations are carried out in the fruit sampling room, in a controlled environment with the aim of accelerating the development of the larvae in the fruit in order to get precise information in a relatively short period of time (Bjeliš et al., 2013).



**Fig. 5.** Tables for collection of larvae from fruits samples (left) and collected fruits in plastic bags during sanitation in peach orchard (right).

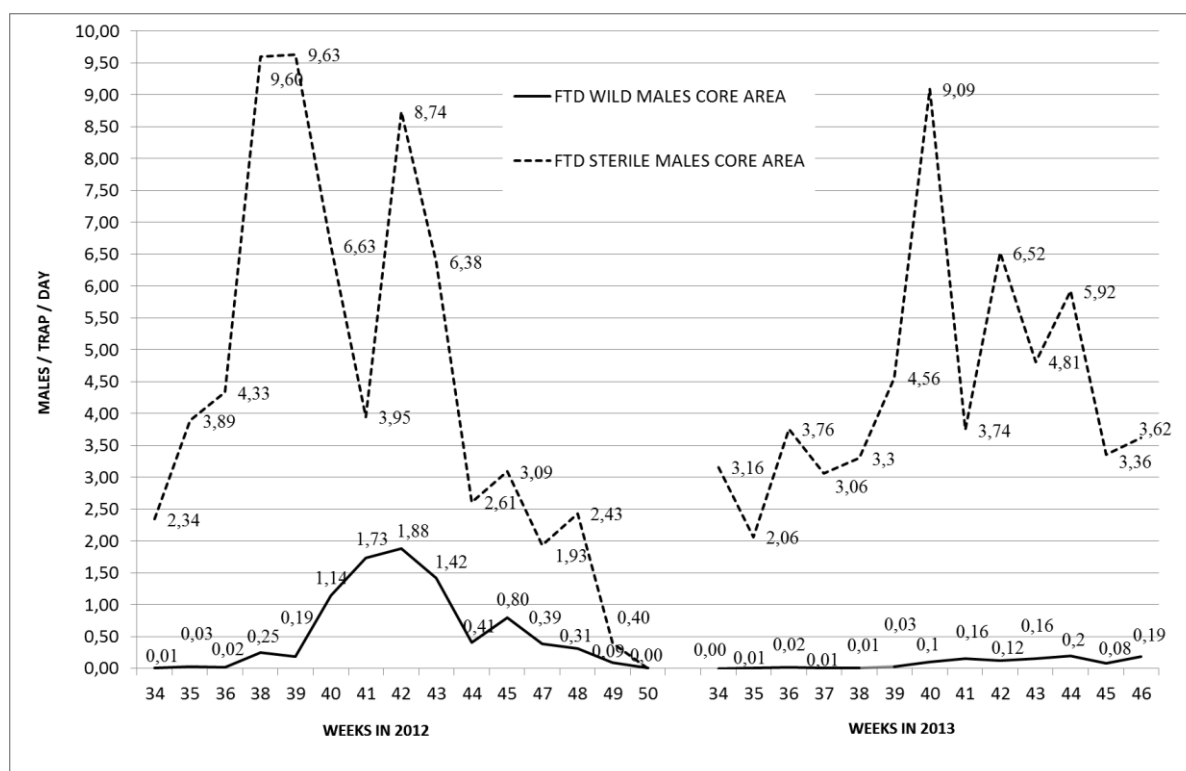
## **Results and Discussion**

The two main parameters used by the Croatian Project in Neretva River Valley to measure the performance of the SIT in the field and suppression efficacy are: Fruit infestation in the main medfly hosts and ratio between sterile males and wild males. Even when there are several important fruit hosts, evaluation of fruit infestation were done on peach (*Prunus persica*), fig (*Ficus carica*) and mandarin (*Citrus reticulata*). The technical index called the “sterile to wild medfly male’s ratio” indicates the level of sterile males competing with the wild males for matings with wild females in the field. This ratio is given by dividing the Flies per Trap per Day (FTD) of the sterile male population by the FTD of the wild male population, which is obtained from the weekly trapping applied in the SIT treated area (FAO/IAEA, 2013). Sterile and wild medfly males FTD obtained is shown in Fig.6 after week 38 when first wild captures were recorded during 2012 and 2013 and ratio between sterile and wild males is shown in Fig.7, being the basis for the effective suppression of the pest using this technique.

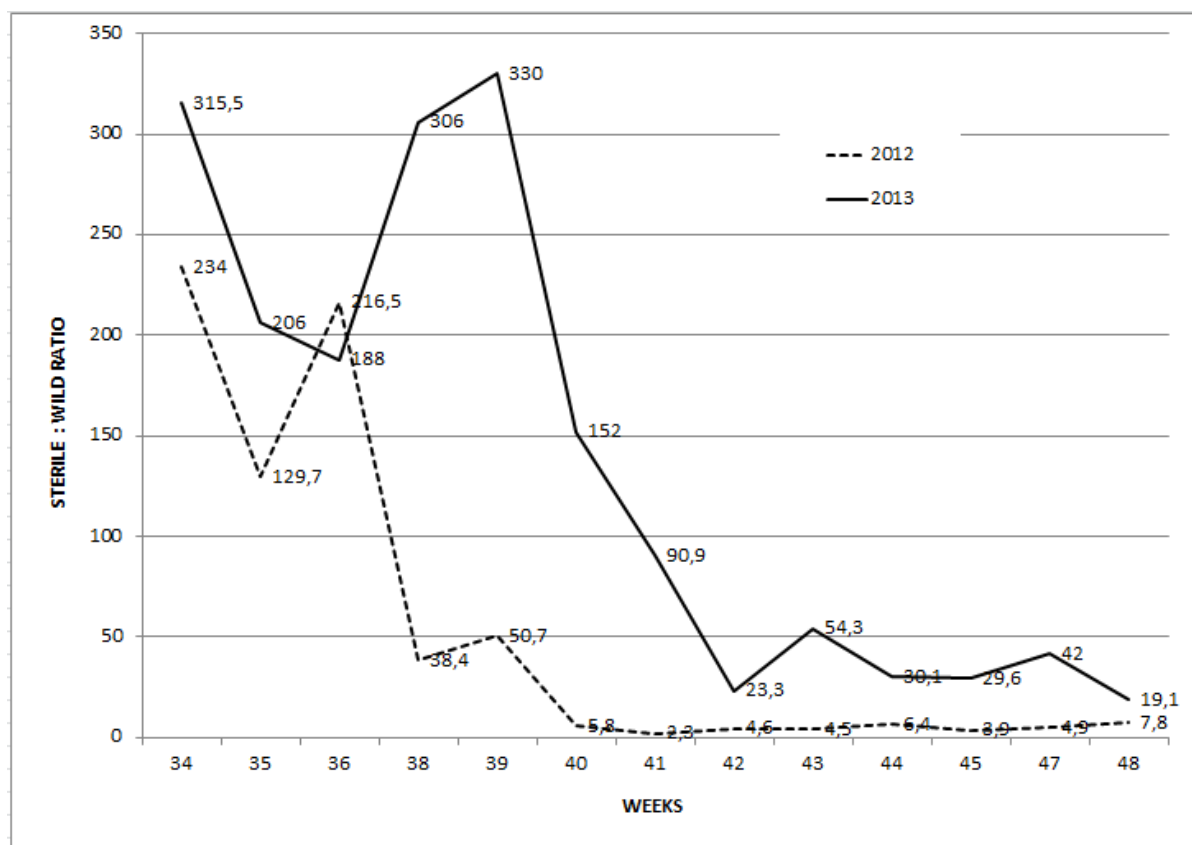
Fig.6 and Fig.7 shows that sooner or later in October there was a significant decrease in the value of the capture of wild and sterile males to 3C bait and reduction of sterile:wild ratio which can be explained due to the autumn weather changes, means falling of the temperatures and frequent rain. An effective and practical way to evaluate the effectiveness of the SIT is comparing infestation levels in fruits of the main host plants in neighboring SIT treated and non-treated areas. Fruit of the main hosts are collected in high numbers and under the same conditions and dates in both areas. The fruits were placed on the mesh over the 1 x 3 meters collection tables for maturation and collection of all the larvae coming out from the fruits for pupation. At the end of the maturation and collection of larvae, the fruit is finally dissected to collect the residual larvae left inside. By counting the medfly larvae from both areas, the infestation level is obtained and given in the form of larvae per kilogram of fruits.

In Figures 8 and 9 the results of the evaluation are given based on samples of peach, fig, and mandarin as the main host fruits that were protected under this SIT project during 2012 and 2013.

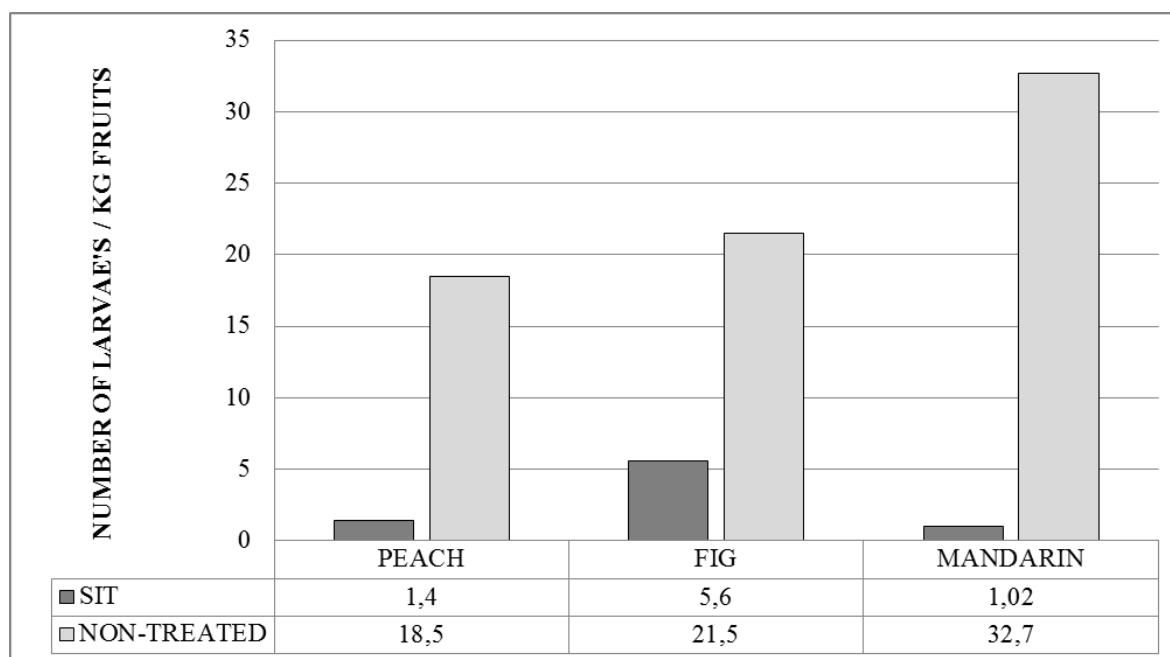
As the export of mandarins from the Neretva Valley is the main economic activity of this region, and the medfly is a limiting factor to increase this activity, a specific evaluation was carried out by sampling fruits of shipments aimed for exports in order to assess infestation levels. Reduced infested shipments provide better conditions for the acceptance of the mandarins in markets with more stringent phytosanitary regulations.



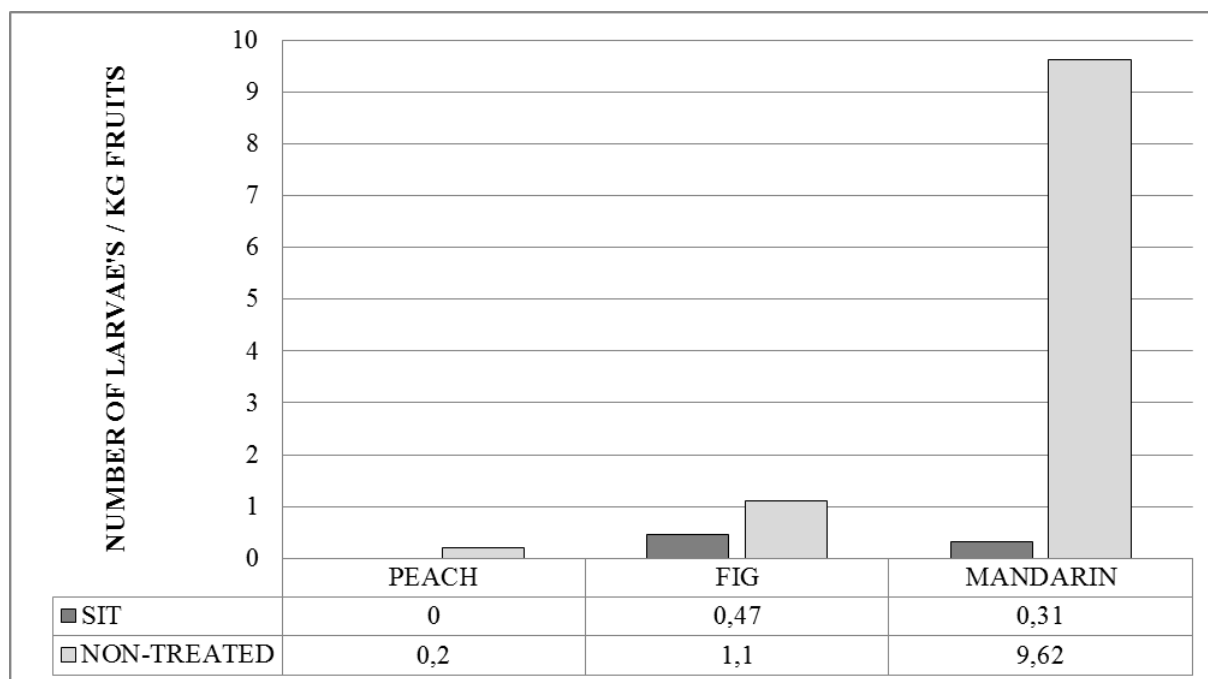
**Fig. 6.** Comparison between sterile and wild medfly males FTD from Tephri Traps baited with 3C lures in area where sterile insect technique was applied during 2012-2013.



**Fig. 7.** Sterile to wild males ratio from Tephri Traps baited with 3C lures in area where sterile insect technique was applied during 2012-2013.



**Fig. 8.** Percent fruit infestation in SIT treated and non-treated area in Neretva Valley during 2012.



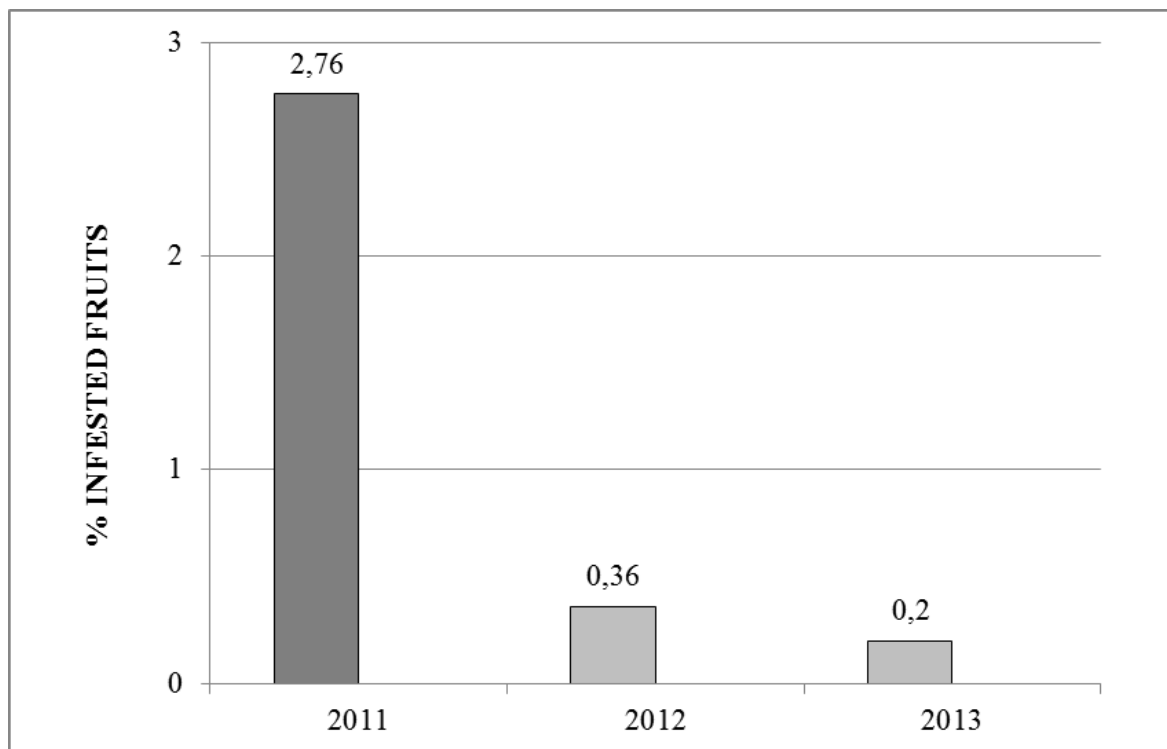
**Fig. 9.** Fruit infestation given in number of larvae per kilogram of fruit in SIT treated and non-treated area in Neretva Valley during 2013.

In Fig.10, the results of this specific sampling are given for 2011 (before expanding program), 2012 and 2013. Results show a significant reduction of infested shipments from year to year. Furthermore, it is important to stress that previous to expanding the program, almost 100% of the shipments contained infested fruits, after expanding of the area, the percentage of infested shipments were reduced to 53,3% in 2012 and 26% in 2013.

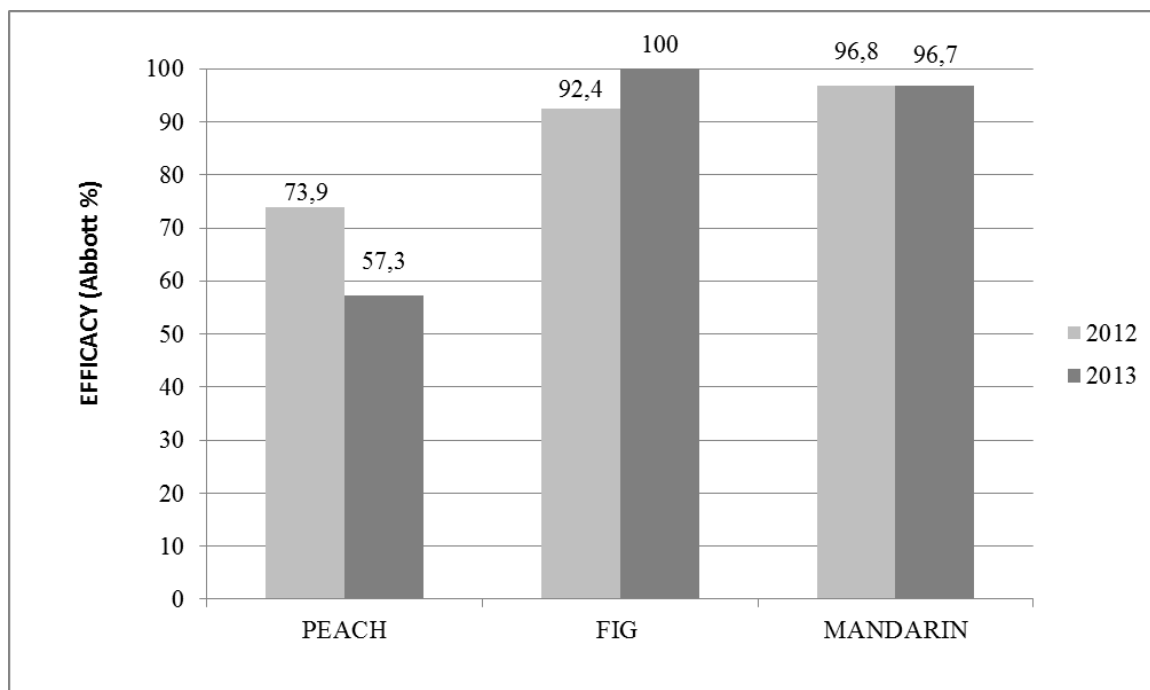
The efficacy of suppression of medfly by integrating SIT to other surveillance and control methods, is shown in Fig.11.

Efficacy was evaluated based on the comparisons of infestation level measured by number of larvae per kilogram of fruits as shown in the Fig.2 for peach, *P. persica*, fig, *F. carica* and mandarin, *C. reticulata*, the three most important crops. The infestation level between the area treated with sterile males and the untreated area at the north part of the valley was used to calculate efficacy. The results of the application of SIT as a species specific and environmentally acceptable method of combating the medfly over an area of 4000 ha, confirms the high effectiveness of the SIT for the suppression of this pest in the ecological conditions of the Neretva Valley. Based on the results, it is to be expected that this IPM scheme will be applied to the entire area of the Neretva Valley including the side of Croatia and Bosnia and Herzegovina in an area-wide approach.





**Fig. 10.** Average infestation of mandarin's fruits by medfly larvae's in export shipments before (2011) and after (2012 and 2103) expanding SIT treated area.



**Fig. 11.** Efficacy of the SIT for suppression of Medfly in Neretva Valley of Croatia.

## Acknowledgments

Authors wish to acknowledge the growers Association for the overall contribution and participation, private packing house at Konzum, Croatia, for the space, fruit samples and to the Joint FAO/IAEA Insect Pests Control Section, Vienna, Austria for scientific and technical support through several Technical Cooperation Projects.

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## **Descriptive analysis of the factors affecting population fluctuation of the Mediterranean fruit fly (*Ceratitis capitata*, Wied.) in coffee areas located in Guatemala and its implications in IPM Strategies**

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### **Abstract**

Since 1975, the Guatemala-Mexico-United States Mediterranean fruit fly Containment and Eradication Programme (Moscamed Programme) conducts control activities to protect the medfly free status in the United States, Mexico and north of Guatemala and to gradually eradicate the pest from Guatemala. This has been possible through a dynamic containment barrier at the Guatemala - Chiapas, Mexico border. In the past seven years (since 2008), the barrier has been gradually moved away towards the east of Guatemala advancing nearly 20,000 km<sup>2</sup> resulting in new pest free and low prevalence areas. Here, we analyzed the influence of climate and host phenology on population fluctuation of the medfly in areas with area-wide SIT releases and implementation of other IPM tools. We also analyzed the implications of population fluctuation on strategic planning and management of medfly populations. The results of data analysis show how medfly populations are influenced by the fruiting phenology of coffee, its main host, as well as by climatic factors mainly temperature and rain. Based on the influence of these factors, two distinctive population fluctuation patterns are observed in Guatemala, one at the Southwest Pacific Coast Region and the other at the North Transversal Strip Region. In order to understand better how these multiple factors interact and affect medfly populations, a more in depth analysis should be conducted using more sophisticated statistical analysis. Results also show that in some years populations are affected by extreme weather conditions associated with the ENSO (El Niño-Southern Oscillation) and La Niña meteorological phenomenon. Drastic unpredictable population fluctuations of medfly, influenced by climate conditions and host phenology, affect the capacity of the programme to maintain adequate sterile: wild ratios required to suppress and



eradicate populations. A 10 year analysis shows that when the sterile: wild ratio has been consistently maintained at 100:1 or above, the pest remains under control and with reduced dispersion over the low prevalence and pest free areas. These ratios correspond mostly to medfly control in a coffee ecosystem, where the pest finds optimum conditions for reproduction and growth. For the Moscamed Programme, understanding the interactions between pest, host and climate is key in designing adequate operational strategies that will allow a more effective medfly containment and eradication effort.

**Keywords:** Mediterranean fruit fly, population fluctuation, programme management, sterile insect technique.

## Introduction

The Mediterranean fruit fly (*Ceratitis capitata* Wied.) is considered to be one of the most destructive agricultural pests because of the direct damage it inflicts to fruit production reducing yields as well as indirect damage from quarantine restrictions imposed by countries that are free from the pest that limit commercialization of fruits and vegetables. This damage is well documented in a number of benefit-cost assessments which have analyzed the potential damage to horticultural industries and the economic returns of programme intervention (Gutiérrez Samperio, 1976; Enkerlin, 1997; Enkerlin, 2005; IICA, 2009). The pest was introduced to Costa Rica in 1955 and spread across the Central American region reaching Guatemala in 1976 (Patton, 1980). Taking into consideration the risks and potential impacts of medfly spread into the pest free areas in northern Guatemala, Mexico and the USA, the governments of the three countries decided to join efforts against this pest by means of signing cooperative agreements in 1975 between Mexico and Guatemala, 1976 between USA and Guatemala and in 1981 between Mexico and the USA. In 2015, the agricultural authorities of the three countries joined together in a Trinational Mediterranean fruit fly Containment and Eradication Programme (Moscamed Programme) that updated the previous bilateral agreements providing the Moscamed Programme a more solid framework looking into the future.

The Guatemala - Chiapas, Mexico Region has about 515,000 hectares of coffee (*Coffea arabica* L.) plantations. The coffee is mostly distributed as a belt along the Sierra Madre of Chiapas mountain range in the Pacific Coast although also available in certain regions north of the country. Coffee is known to be the native host of *C. capitata* in its presumed origin of sub-Saharan north eastern Africa. This coffee ecosystem provides *C. capitata* suitable habitat for rapid population growth and dispersal. Since 1975, the Guatemala-Mexico-United States Moscamed Programme maintains control over the medfly populations to contain its spread towards North America and eradicate the pest from Guatemala (Hendrichs et al., 1983; Villaseñor et al., 2000; Gutiérrez Ruelas, 2013). This has been possible through managing a dynamic containment barrier which operates at the Guatemala - Chiapas, Mexico border. In the past seven years, the barrier has been gradually moved towards the east, advancing nearly 20,000 km<sup>2</sup> and resulting in new pest free and low prevalence areas along the border of

Guatemala and the state of Chiapas in southern Mexico (Programa Moscamed, 2013a).

The objective of this paper is to provide a preliminary insight on how host phenology and climate influence the population fluctuation of the medfly in Guatemala and how changes in population trends affect the strategies and tactics used by the Moscamed Programme to manage the pest. It builds on previous studies that were used as the basis for the design and implementation of a programme strategy known as gradual advance programme (Midgarden & Lira, 2008; McGovern et al., 2008). It also utilizes valuable knowledge of a number of highly experienced professionals that have worked for years in the Moscamed Programme.

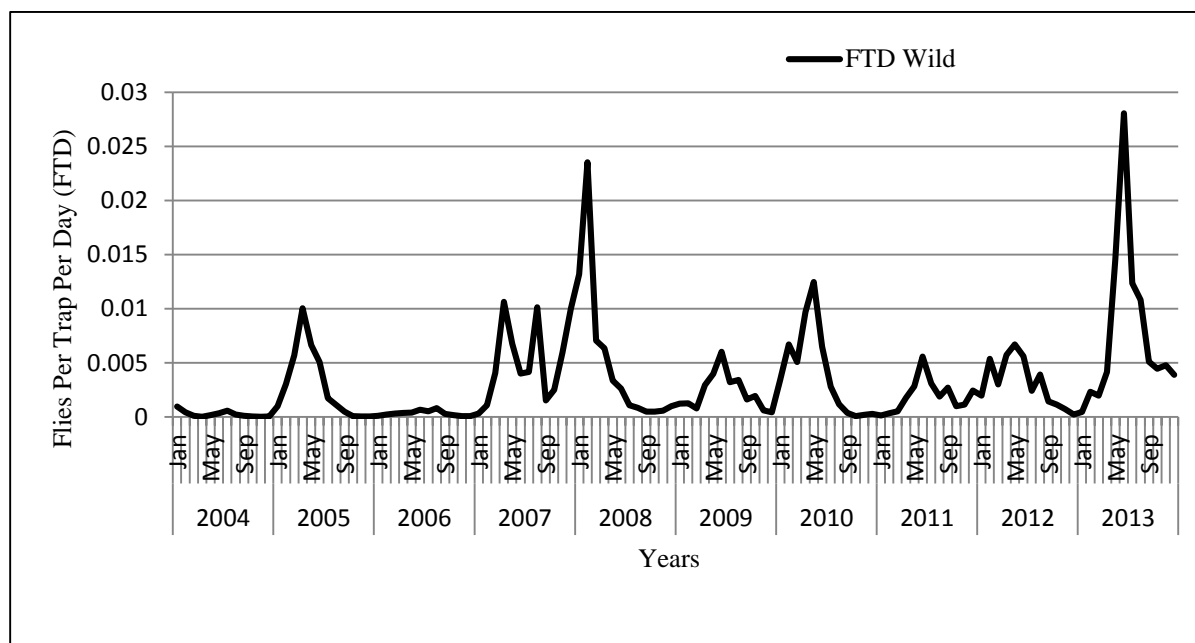
## Methods

Preliminary descriptive analysis of the effects of host phenology, normal climate (temperature and rainfall) as well as extreme climate associated with the ENSO (El Niño-Southern Oscillation) and La Niña weather phenomenon were conducted to observe its effects on medfly population fluctuation. Ten years (2004-2013) of historical trapping data of the Moscamed Programme were summarized as the average number of flies per trap per day (FTD) (IAEA, 2007). The number of traps operated by the programme during this time period was in average 16,000 traps per year. This trapping network covered an area that ranged approximately from 60,758 to 80,526 km<sup>2</sup>. During this time period, medfly populations were subjected to control actions through an area-wide IPM including a sterile insect technique (SIT) component. The relationships between temperature, rainfall and coffee prices on medfly population abundance were explored using simple correlation analysis. The relationship between medfly population abundance and the sterile to wild ratio was also assessed with simple correlation analysis. This type of analysis was selected in order to observe the results of the interaction between two variables and to provide a first approximation.

## Results and Discussion

### *Factors Affecting Medfly Population Fluctuation*

The medfly population fluctuation in Guatemala and Chiapas is strongly associated with the phenology of coffee, its primary host. Both the medfly and coffee cycles are shaped by climate conditions, mainly temperature and in a lesser degree rainfall (Midgarden & Lira, 2008). The spatial and temporal population fluctuation as measured by trapping for medflies within the programme area is well known (Programa Moscamed, 2013, Fig.1). At least two distinctive spatial and temporal patterns associated with phenology of coffee and prevailing climatic conditions are recognized. One occurs in Southwest Guatemala (SW Region), along the Pacific Coast, and the other in Northern Guatemala in a region known as the North Transversal Strip (NTS Region) where coffee is cultivated in the highlands around the cities of Coban and Barillas.



**Fig. 1.** Mediterranean fruit fly population fluctuation in Guatemala.

## SW Region

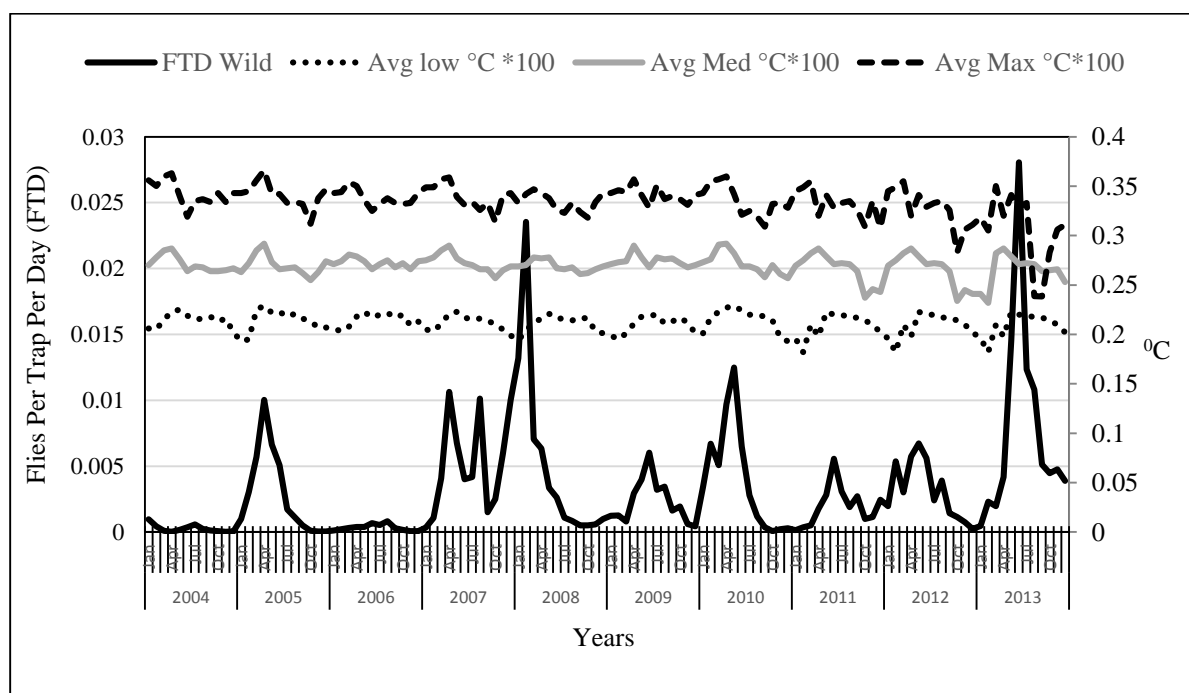
### *Pest-Host Interaction*

Coffee is a crop that demands high volumes of water and grows (thrives) in subtropical-temperate environments at altitudes that range between 400 and 2000 m.a.s.l. In Southwest Guatemala and the Soconusco region of Chiapas, initial erratic rains in April trigger flowering and continuous rains are required for fruit setting and maturation of coffee berries. Moreover, ripening of coffee berries is affected by temperature which, in turn, is associated with altitude. Ripening starts in June at lower altitudes (400 to 600 m.a.s.l) extending to March and April of the following year at the higher altitudes (1800 to 2000 m.a.s.l). Although medfly populations are present throughout the year in the infested coffee production areas in Guatemala, population buildup (measured by adult trap capture) starts in January at higher altitudes in the Southwest Guatemala at the peak of the coffee berry maturation period and harvest, reaching its maximum population numbers between April and May, one to two generations after coffee harvest and during the months of the year with the highest temperatures and lowest rain fall. During these months, with very few coffee berries left in the field and a high population density, medfly adults switch from a non-dispersal to a dispersal behavior moving from the coffee growing areas to search for alternate hosts such as peaches (*Prunus persica* L.), pears (*Pyrus communis* L.), mandarin (*Citrus reticulata* L.) and sour orange (*Citrus aurantium* L.) present within or nearby the coffee plantations at mid to high altitudes (600 to 2000 m.a.s.l). They may switch as well to tropical host species such as guava (*Psidium guava* L.), caimito (*Chrysophilum cainito* L.), tropical almond (*Terminalia catappa*, L.) and mango (*Mangifera indica* L.) located at lower altitudes (100 to 500 m.a.s.l) along the Pacific Coastal line (Midgarden & Lira, 2008). After the population peaked in April and May, a slight decrease is

observed starting in June reaching its lowest levels in November and December, even despite the fact that abundant coffee berries are again available for population increase in the field (Fig.1). This time period coincides with the lowest annual temperatures (7-20°C) and as a result, the pest experiences longer life cycles. In addition, this is the time of the year with the highest rainfall which could also have an effect on regulating population growth rate (Manrakhan & Lux, 2006), as is discussed in greater detail in the next section.

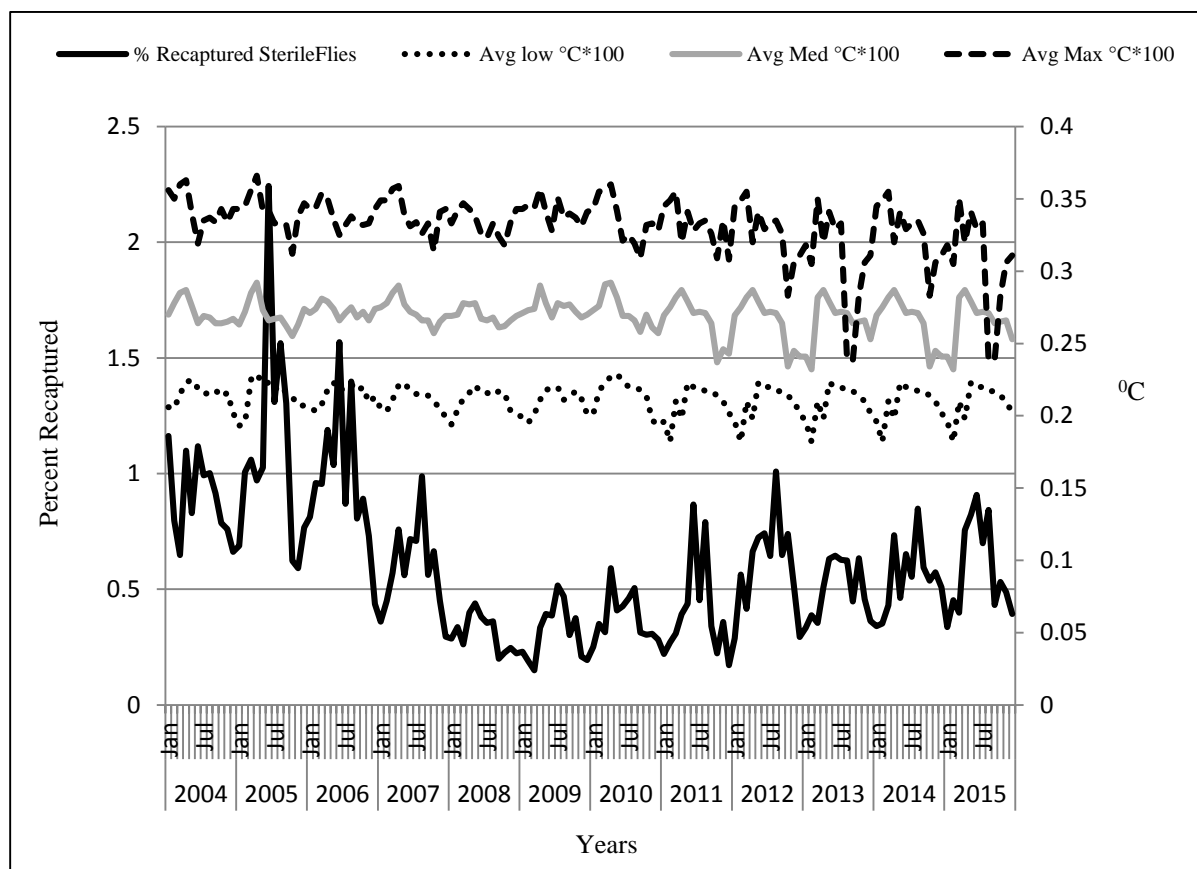
#### *Pest-Climate Interaction*

In this region, climate is classified as tropical-subtropical. However, prevailing conditions are affected by altitudinal strata and significant variations in temperature and rainfall that affect medfly population fluctuations are observed. Lower temperatures ranging from 6 to 20°C occur at higher altitudes from 400 to 1850 m.a.s.l. during the months of November to February. Such low temperatures increase the length of medfly life cycle affecting population growth rate. Population increase is observed starting in January and peaking in April and May when minimum and maximum temperatures increase to 22 - 24°C and 34 - 37°C, respectively (Fig.2).



**Fig. 2.** Mediterranean fruit fly population fluctuation in relation to temperature in Southwest Guatemala.

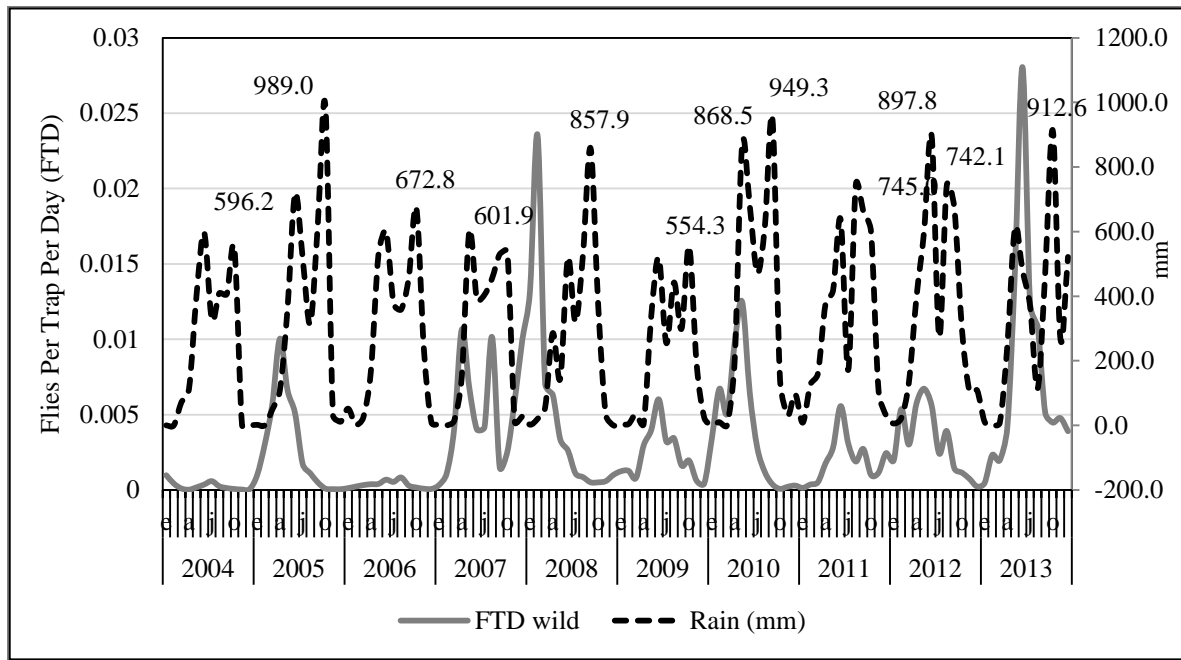
However, low temperatures might also affect the mobility of flies as well as trap efficiency, therefore, trap capture. This can be observed with the release and recapture of sterile flies as the amount of recaptured flies significantly decreases during the winter months and increases during the summer months, even when no major difference is observed in the amount of released sterile flies (Fig. 3).



**Fig. 3.** Mediterranean fruit fly released-recaptured sterile flies in relation to temperatures.

Data derived from records of rainfall over 20 years shows that the rainy season extends from May to October. A first peak occurs between May and June, with a marked reduction in rainfall during a small dry period, which occurs in July. A second peak is observed during August and September which are the months with the highest rainfall ([http://www.insivumeh.gob.gt/meteorologia/mapa\\_estaciones.htm](http://www.insivumeh.gob.gt/meteorologia/mapa_estaciones.htm)). In this Southwest region medfly population reaches its highest peak between April and May starting its decrease during the month of May and June due to the lack of coffee berries available for oviposition in the field. A drastic population reduction is observed during the months of August and September when the heaviest rainfalls are observed reaching its lowest numbers in November and December when temperatures drop. This might be an indication of the detrimental effects of rain over medfly populations in combination with other factors, mainly temperatures. Some authors indicate that heavy rains may wash away from leaves natural adult fly foods such as bird feces and honeydew, reduce adult fly activity due to increase cloud cover and reduction of light and increase pupae mortality due to soil saturation (Fig.4) (Manrakhan & Lux, 2006). Yet, in spite of this, rainfall is considered to be a secondary factor affecting the population fluctuation of medfly compared with host phenology and temperature.





**Fig. 4.** Mediterranean fruit fly population fluctuation in relation to rain in Guatemala.

## NTS Region

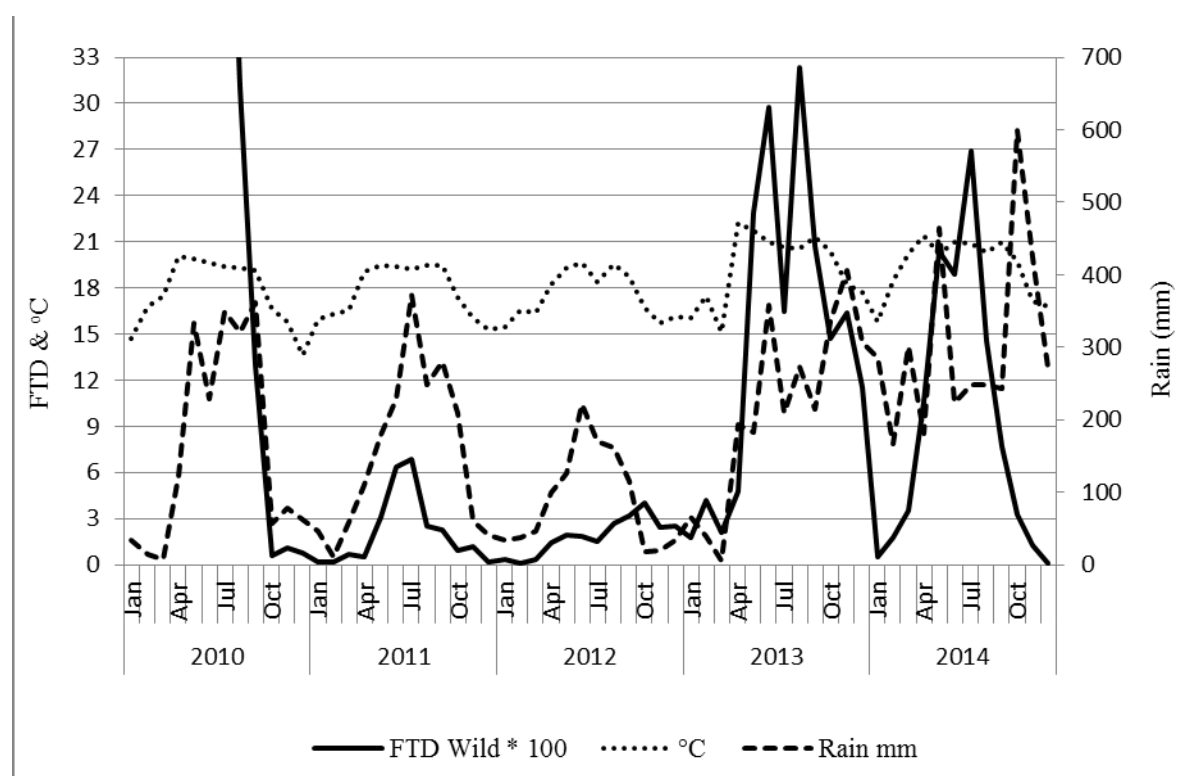
### *Pest-Host Interaction*

In this region, coffee phenology follows a different pattern, as well as the medfly population fluctuation. Coffee starts ripening in October and harvest ends in February compared to a much extended maturation and harvest period at the coast where ripening starts in June at low altitudes (400 m.a.s.l) and harvest ends in March-April at the highest altitudes (1800 to 2000 m.a.s.l), as explained above. Coffee maturation in the NTS Region occurs at the time of the year when temperatures are the lowest with minimum temperatures fluctuating between 8 and 15°C. Therefore in this region, the most important factor regulating populations is temperature as compared with the SW Region where, both, host availability (coffee) and temperature have major influence on population fluctuation. During the coldest months of the year when coffee berries have ripened and are available for infestation (October to February), medfly populations are at their lowest levels. Therefore, temperature becomes the critical factor affecting medfly population fluctuation.

### *Pest-Climate Interaction*

In this region, climate is classified as subtropical-temperate with more drastic variations in temperatures depending on the altitude compared to the previous region. In this case, coffee plantations are concentrated in the area of Coban and Barillas predominantly at high altitudes (800 to 1300 m.a.s.l.) and therefore ripening and availability of coffee berries for infestation occurs in a more discrete area and period of time. In this case, medfly populations start to build-up in May and June (compared to January in the SW Region) reaching its maximum peak in July (compared to April and May in the SW Region) with a clear population drop

starting in August reaching lowest numbers in October (compared to November and December in the SW Region) (Fig.5). It is interesting to note that medfly populations are observed in this region mostly from June to September, during the rainy season and when only very few coffee berries are present in the field since coffee maturation and harvest occurs from October to February. In this case, low winter temperatures may have a more significant effect and play a more relevant role in regulating medfly populations than availability of coffee berries, contrary to what is observed in the SW Region where, in general, temperatures are milder and maturation phenology of coffee has a stronger effect on the population fluctuation of the pest (Fig.5).



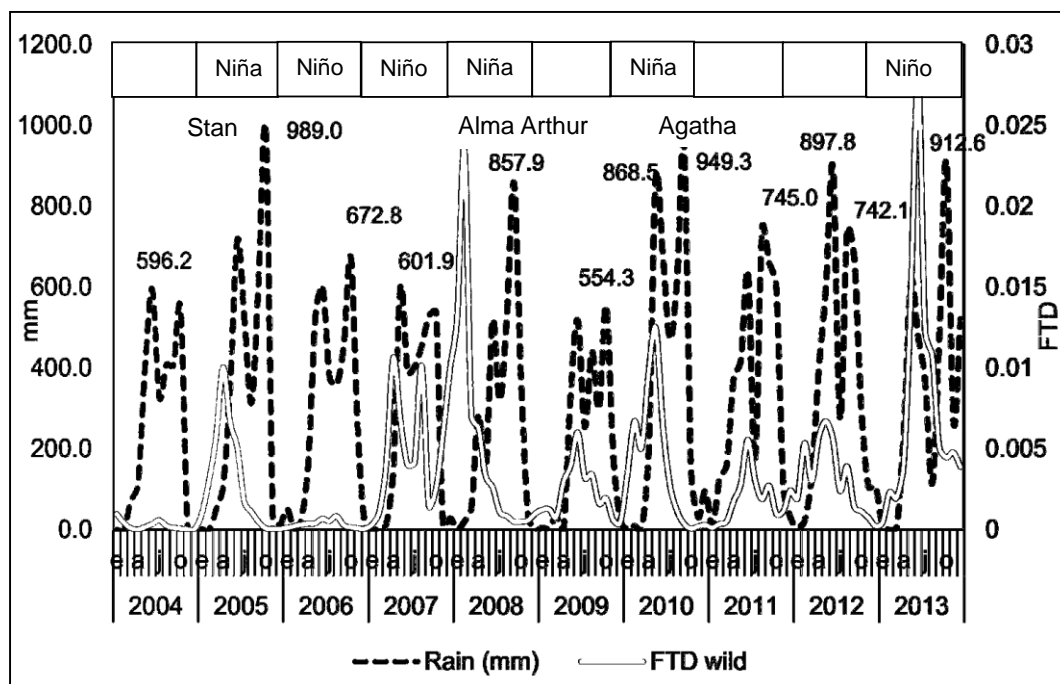
**Fig. 5.** Mediterranean fruit fly population fluctuation in the NTD Region (Coban) in relation to temperature and rain.

The association between the variables medfly abundance and temperature showed a significant positive but moderate correlation ( $Rho = 0.3249$ ,  $P = 0.0004$ ,  $df = 116$ ). As discussed above population reductions are observed during the colder months of the year. Whereas, the association between medfly abundance and rainfall showed a significant but low correlation between variables ( $Rho = 0.1827$ ,  $P = 0.0487$ ,  $df = 116$ ).

### *Effects of extreme meteorological conditions on medfly population fluctuation*

As described in previous paragraphs, the major abiotic factor affecting population fluctuation of medfly is temperature and in a lesser degree rainfall. The later, are exacerbated when associated with the ENSO (El Niño-Southern Oscillation) and La Niña meteorological phenomenon (Herrera, 1998). As with any other insect pest, temperature is a critical factor affecting the length of the biological cycle and thus the number of generations per year and size of the population. In El Niño years, weather conditions favor warmer temperatures and a higher population growth rate resulting in higher than average trap captures (population explosion) and high population invasive pressure over the low prevalence and free areas. Whereas, in years with contrasting unfavorable weather conditions for the pest known as La Niña years, temperatures are lower and often generate hurricanes or tropical storms which can cause heavy flooding (more than 24 hours of rainfall) resulting in a suppression effect over the pest populations. Two consecutive El Niño years will exacerbate the effects, as occurred in 2006 and 2007. However, high populations can be drastically reduced if an El Niño year is followed by a strong La Niña year as can be observed in years 2005 - 2006 and 2010 - 2011.

The effect of two major tropical storms classified as hurricanes, namely Stan that occurred in 2005 and Agatha that occurred in 2010 can be observed in Fig.6 where heavy rains and flooding caused a knockdown effect over the medfly populations (Auclair et al., 2008). Stan with eleven days duration (27 September to 7 October 2005), the most intense in the last decade, had a major suppression effect on pest populations, which lasted around 14 months. Agatha with five days duration during the month of May, in addition to the prolonged rainy season in October (the highest volume of accumulated rain in the whole 10 year study period), caused a significant suppression effect of almost eleven months.



**Fig. 6.** Wild medfly population fluctuation and rainfall associated to El Niño and La Niña (including tropical storms and hurricanes).

The 2008 storms (Alma & Arthur) were short (only two days) and no suppression effect was observed. The effects of major tropical storms and hurricanes on medfly population have also been documented in previous years. In 1998, the hurricane Mitch, the most disastrous in modern age with a 5 category, 14 days duration (from 21 October to 5 November) and wind speeds of 290 km/hr drastically reduced the pest which had spread to the northern region of Guatemala and Chiapas for the following months. This, in conjunction with other meteorological events impeded medfly populations to recover until 2002. Other Tropical storms such as Katrina in 1999, Keith and Chantal in 2000 and Iris and Michelle in 2001 did not affect pest abundance.

### **Other factors affecting medfly populations**

Medfly populations are also affected by externalities, which are indirect factors that can have an impact on the medfly population growth rate and density. The main externalities are coffee prices and coffee yields.

#### *Coffee prices*

Coffee prices are set by the international markets based on supply-offer. Guatemala has over 300,000 hectares of cultivated coffee and produces over 200,000 tons of coffee per year with a value that fluctuates between US \$800 million and 1 billion per year. Since the coffee industry in Guatemala is mostly export oriented, international prices directly affect sales. Extreme low prices from an excess supply resulting from, for example, surplus production in Brazil will have an impact on volumes of coffee to be exported from Guatemala. The internal coffee market has a low elasticity, thus, coffee volumes which are not exported will not be sold in the local market. Under an extreme situation some coffee growers will choose to leave at least part of the production unharvest. This creates a situation where large amounts of coffee berries are left in the field as substrate for medfly oviposition and continuous reproduction as well as coffee berries which are not processed in the coffee mills preventing larvae mortality by mechanical action during processing. This scenario would seem to result in huge amounts of additional medflies in the field which would represent a big challenge for the Moscamed Programme as it would translate into more pest pressure for the low prevalence and pest free areas in Guatemala and Chiapas. Such situation has been occasionally observed in the past 35 years in Mexico and Guatemala although there is no conclusive evidence of its effects. Here, the association between medfly abundance and coffee prices showed no significant correlation ( $Rho = 0.0041$ ,  $P = 0.96$ ,  $df = 116$ ). Nevertheless, this factor should be taken into consideration when forecasting medfly population trends in a particular year.

#### *Coffee yields*

A similar effect can be expected in years of low coffee yields. Very low yields can result from extreme climate conditions such as a late or early frost, heavy rains and high speed winds from hurricanes or from the presence of a pest causing severe damage such as an outbreak of

the coffee rust (*Hemileia vastatrix*, Berk.). Although this situation due to reduced supply could increase coffee prices in the international market and benefit the coffee industry overall, the affected countries will suffer. Some low-income growers might decide to leave the crop in the field since the hand labor required to harvest the crop would result more expensive than the revenues that could be obtained from selling the crop. As in the case of low coffee prices, although there is no conclusive evidence documenting the effects of low coffee yields on medfly populations, this factor should be considered when trying to predict population trends.

### **Factors affecting pest movement**

As shown in the previous sections, medfly population fluctuation as well as temporal and spatial distribution is intrinsic to variations in climate (temperature and rainfall), and primary host phenology (mainly coffee berries). Although, through this study other external factors such as coffee prices and yields showed not to have a significant effect on population fluctuation, a more detailed analysis should be conducted in order to draw final conclusions.

In years where prevailing conditions favor an increase in medfly population densities, pest movement from highly infested areas can inflict high invasive pressure over low prevalence areas (LPA) and pest free areas (PFA). Therefore, detailed understanding of medfly movement and characterization of pathways is key to find appropriate strategies for pest control and for protection of LPA and PFA. Medfly movement can be separated into natural and artificial movement.

For long distance natural movement, insect pests including medfly, may use dominant wind currents that can disperse adult flies tens of kilometers away. In a recent release-recapture study conducted in Guatemala using marked sterilized medflies, it was shown that most adult flies disperse short distances in the order of hundred meters, however, some can disperse long distances, up to 51 km away from the release point (Villatoro, 2013). Midgarden and Lira, 2008, discuss the possibility of medfly adults spreading, after coffee harvest, from the coffee growing regions in the highlands of Guatemala to the Pacific Coastal region of free areas in Guatemala and Mexico, aided by wind currents probably trade winds typically blowing from northeast to southwest. Therefore, assessment of trade wind patterns and dominant winds that are generated from orographic effects is important at a macro level for characterization and assessment of pest pathways and interpretation of the overall spatial distribution of the pest.

It has been shown that artificial movement of the pest plays a major role in its dispersal from infested areas into LPA and PFA. This happens when infested fruits are carried by merchants in small, medium and large volumes to be sold in fruit markets located in communities within the LPA and PFA. In the case of Guatemala, coffee berries (the main medfly host) are transported in large volumes from infested areas to coffee processing plants some of which are located in PFA. It may also occur when small amounts of infested fruit are carried as food by illegal migrant workers from Guatemala and the rest of Central America travelling long distances across Mexico and the USA. Historical data of confiscated host fruit at the programmes quarantine checkpoints, has allowed the programme to assess with certainty



which fruit species have the highest risk of pest movement as well as the time of the year when these fruits are transported. Historically, the commercial hosts that have resulted in the highest number of medfly larvae and thus considered to be of higher quarantine risk are in order of importance: Coffee berries, peach, pear, guava, mandarin and mango (Table 1). Other less commercial hosts which represent high risk are: white sapote (*Casimiroa edulis* La Llave & Lex), sour orange, loquat (*Eriobotrya japonica* Lindl), and tropical almond (*Terminalia catappa* L.). The time of the year when these fruits are confiscated at the quarantine checkpoints varies according to the fruiting season of each species. Early in the year (February to May) coffee is the host that yields the highest numbers of medfly larvae. Early-midyear (March to June), it is peaches and pears, whereas, later in the year (August to December) most medfly larvae are found in mango, mandarin and guava. This is considered to be the main pathway for pest dispersal to the medfly free areas in northern Guatemala and northeast Chiapas. A correlation analysis has indicated that 74% of the medfly transient entries into this PFA is related to the distance of the leading edge of the infestation in Guatemala to the PFA probably due to natural dispersal of the pest. The remaining 26% is due to other factors, including, predominantly movement of infested fruit through quarantine checkpoints and uncontrolled side roads (Enkerlin et al., 2015).

### **Implications in Programme Management**

It is clear that effective management of medfly populations requires a clear understanding of the influence of the main biotic and abiotic factors affecting population fluctuations. This preliminary analysis has shown that the interactions between pest, availability of the primary fruit host (coffee), temperature, and rainfall have influence on population fluctuation as well as on spatial and temporal distribution. When the combined effect of these factors favors pest population growth rate, the risk of pest dispersal through dominant winds and infested fruit hosts is exacerbated.

This has major implications in the programme's strategic planning and management. From this data and maintaining current programme conditions (i.e. programme budgets are kept with no variations and there is no breakthrough in technology), three main scenarios for medfly strategic planning and management can be drawn (Table 2): 1) The combined effects of a hot and dry year (el Niño year) and normal to higher than average coffee yields will result in an increase of population and high population pressure over the LPA and PFA. In these years the programme should favor the protection of PFA through a containment strategy and with no or a limited gradual advance depending on the pest pressure; 2) In contrast, the combined effects of lower than average temperatures and presence of heavy rains concentrated in short periods of time (typical rains that occur during tropical storms and hurricanes) and normal or below average coffee yields, will result in a reduction of population density and lower pest pressure towards the LPA and PFA. In these years the programme's strategy should shift from a containment strategy to one with a high rate of advance; and 3) Which is the normal scenario with average temperatures, rainfall and coffee yields and therefore an average population density and pressure over the LPA and PFA. In these years

the programme should be able to achieve a moderate gradual advance rate.

### IPM tactics

Understanding the interactions among pest, host and climate and how this affects the fluctuation of medfly populations is fundamental, not only for strategic planning, but also for the effective application of the available control and surveillance tools and the tactics used as part of an IPM programme against the pest.

**Table 1.** Mediterranean fruit fly larvae intercepted at quarantine checkpoints in Guatemala.

Host	Year											Total
	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	
Coffee	365	33	443	437	1,066	184	2,044	235	121	31	71	5,030
Star Apple		30	29	120	2	18	12					211
Guava	58	63	19	18	4	171	10	10			12	365
Sour Orange	2			106			95					204
Mandarin	15	17	9	82	23	6	7		5		1	165
Sweet Orange		21	6	26	16		10	6		7		92
Mangoe	6	64		39	2	2	3			19	12	147
Pear	172	500	77	111	574	192	83		2	25		1,736
White sapote		2	16	651	7	9					8	693
Lime					4					3		7
Peach	15	1,412	102	754	137	155	962	45	13	484	40	4,119
Rose Apple						77						77
Tropical Almond		28	28	2		44	6	20		2		130
Apple		61		12					1			74
Icaco						4				5		9
Soursop		121										121
Sapodilla		44		12						2		58
Medlar	11	10				133	37			8		199
Mombin									1			1
Rose Apple		104										104
Apricot	2				7	4						13
Plum						2					1	3
Quince	4											4
Crabapple											1	1
<b>Total</b>	<b>650</b>	<b>2,510</b>	<b>729</b>	<b>2,370</b>	<b>1,842</b>	<b>1,001</b>	<b>3,269</b>	<b>316</b>	<b>144</b>	<b>586</b>	<b>146</b>	<b>13,563</b>

As has been shown, favorable years for the pest will result in an increase in population density which may translate into higher dispersal rate and thus in an increase in pest entries

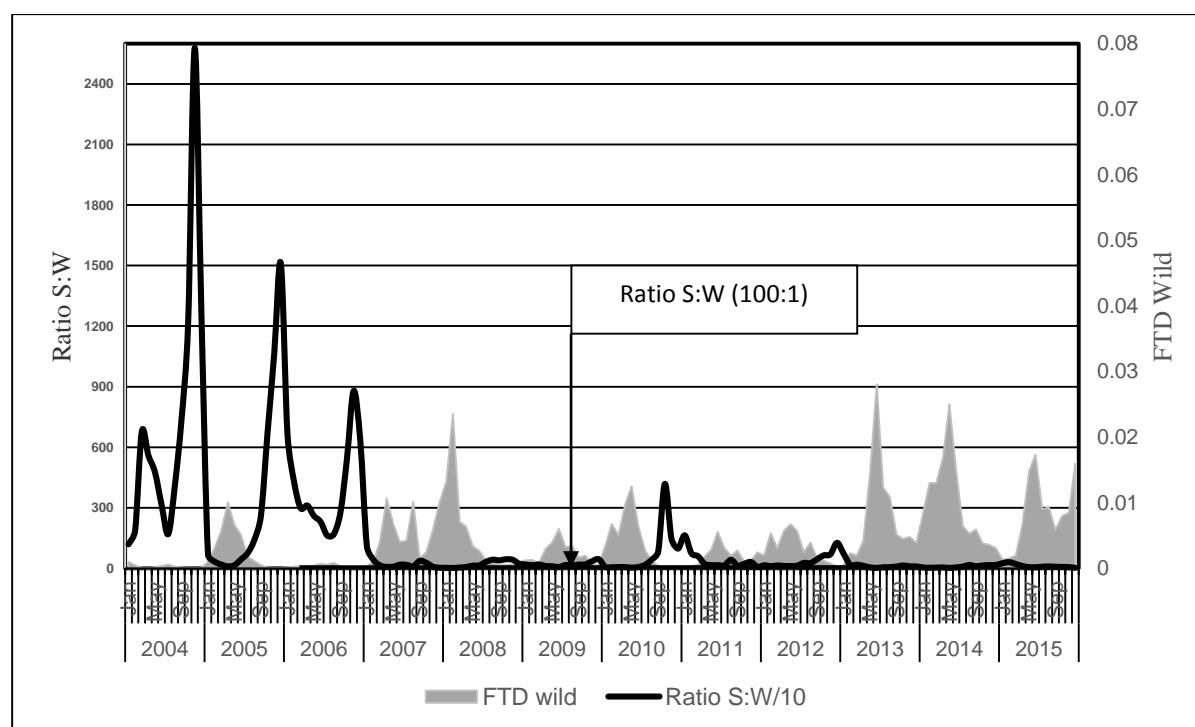
into PFA and LPAs. Unfavorable years for the pest will result in the opposite, that is, in lower pest pressure. Therefore, the tactics: size of the intervention areas, the intensity in the application of the control and surveillance tools and the timing of the application will have a direct relation with favorable or unfavorable years for the pest.

**Table 2.** Suggested Mediterranean fruit fly management strategies.

Scenario	Temp (°C)	Host (Yield)	Rain (mm)	Extreme weather	Externalities (Coffee prices, etc.)	Strategy
Normal	Average	Average	Average	No	No	Gradual advance –
Extreme – bad	High (Hot)	High	Low (Dry)	Yes (Niño)	Yes (Low prices)	Medium rate Containment/ Low rate of gradual advance (1992, 1998, 2002, 2007) <sup>1</sup>
Extreme – good	Low (Cold)	Low	High (Saturation)	Yes (Niña)	No	Gradual advance – High rate

<sup>1</sup>This scenario occurred during the indicated years.

An analysis performed over 10 years of data on sterile to wild ratios showed a moderate to high correlation ( $Rho = -0.6704$ ,  $P = 0.0001$ ,  $df = 116$ ). It also indicated that when the ratio is 100 to 1 or above, the pest remains under suppression with reduced dispersal over the LPA and PFA and eventual eradication. On the contrary, when the ratio is below 100:1, the suppression effect decreases and the dispersal over the LPA and PFA increases (Fig.7).



**Fig. 7.** Impact of SIT on medfly populations with sterile to wild ratios above or below 100 to 1, Moscamed Programme Guatemala.

These findings are consistent with the sterile to fertile ratios obtained in a field experiment conducted in large cages where different proportions of sterile and wild medflies were released using coffee as the host and aimed at assessing the ratios required for population suppression and eradication (Rendon, 2004). It is also consistent with the sterile to wild ratios recommended by FAO for containment, suppression and eradication of medfly populations (FAO/IAEA, 2007). It is important to note that these figures correspond for the most part to the coffee ecosystem, where medfly populations find optimum conditions for reproduction and growth. The ratios may be quite different depending on the prevailing host and climatic conditions.

## Conclusions

For the Moscamed Programme, understanding the interactions between pest, hosts and climate has been key in designing adequate operational strategies allowing a more sustainable management of the dynamic containment barrier.

Drastic population fluctuations of medfly influenced by extreme climatic conditions triggered by El Niño and La Niña meteorological phenomenon and host phenology, affect the capacity of the programme to manage medfly populations. This includes the sterile to wild ratios required to suppress and eradicate populations. A sterile to wild ratio of 100 to 1, has shown to suppress and eradicate medfly populations in coffee, its primary host, and in an environment which greatly favors reproduction rate of the pest.

In order to understand better the interactions between all variables affecting population fluctuation, more sophisticated statistical analysis are required. This would allow developing predictive models to improve medfly management such as taking advantage of extreme events that negatively impact the medfly population applying programme resources to advance into typically difficult areas while the populations are low.

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Mosqueta, Municipio de Barillas. Huehuetenango. Technical report Moscamed Program Guatemala. Ciudad de Guatemala.

## **Detection of *Bactrocera dorsalis* (Hendel) in Mauritius and rapid response**

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### **Abstract**

The first detection of *Bactrocera dorsalis* in Mauritius was in 1996 and it was successfully eradicated. The second detection was on 08 March 2013 in an orchard in the north of the island where one male *B. dorsalis* was caught in a methyl eugenol baited trap. Early detection was possible owing to the on-going fruit fly surveillance programme since 1994. The day following a detection in 2013, the protocol for eradication of *B. dorsalis* was implemented: declaration of a quarantine area of 5 km radius from the detection site, placement of dry and wet traps, application of protein bait sprays, placement of male annihilation technique devices, fruit stripping, fruit clean up, disposal of infested fruits, and collection and incubation of fruits and vegetables in a lab room. Four male flies were detected in the north during the months of March and April 2013. The eradication measures were maintained for a period of two months following the last detection. Since there was no detection in July 2013, the control operations were stopped while fruit fly surveillance was maintained for a further period of 12 weeks. *B. dorsalis* has now been declared eradicated in the north. However, in April 2013 *B. dorsalis* was detected in a methyl eugenol baited trap in the western part of the island. As a result, the protocol for eradication has been initiated. The last detection in the west dates back to 22 November 2013. The eradication measures were maintained for another period of four months. Since there was no detection of *B. dorsalis* in traps or from incubated fruits, all treatments were stopped in March 2014. However, surveillance was maintained for a further period of 12 weeks. Since there has been no *B. dorsalis* detection, Mauritius was declared free from this pest in June 2014.

**Keywords:** *Bactrocera dorsalis*, eradication, male annihilation technique, methyl eugenol, surveillance.

## Introduction

Fruit flies of the family Tephritidae are some of the most destructive and important pests of fruits and vegetables worldwide. Economically important fruit flies of fleshy fruits in Mauritius are, in order of importance, the peach fruit fly *Bactrocera zonata* (Saunders), the Natal fruit fly *Ceratitis rosa* (Karsch), the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and the ber fly *Carpomya vesuviana* Costa. Preferred cultivated hosts for the first three species are mango *Mangifera indica* L., guava *Psidium guajava* L., peach *Prunus persica* (L.) Batsch, loquat *Eriobotrya japonica* (Thunb.) Lindl., water apple *Syzygium samarangense*, citrus *Citrus* spp., and custard apple *Annona reticulate* L., while the most heavily attacked wild fruits are Indian almond *Terminalia catappa* (L.) Ridley. *C. vesuviana* is specific to jujube *Ziziphus jujube* Lam. (Sookar et al., 2008). Fruit flies attacking vegetables in Mauritius include the tomato fruit fly *Neoceratitis cyanescens* (Bezzi), which infests tomato *Solanum lycopersicum* L., the Ethiopian fruit fly *Dacus ciliatus* (Loew), the Indian Ocean cucumber fly *D. demmerezi* (Bezzi) and the melon fly *B. cucurbitae* (Coquillett), which infest cucurbits (Sookar et al., 2012).

The Oriental fruit fly, *B. dorsalis* (Hendel) was detected for the first time in Mauritius in 1996 and it was successfully eradicated in 1999 (Seewooruthun et al., 2000).

Recently, Schutze et al. (2015) reported that *B. dorsalis*, *B. invadens* and *B. papayae*, represent the same species. This pest has spread across most of the sub-Saharan African region and currently reported from 24 countries including the Comoros Island and Madagascar. *B. dorsalis* is considered to be world's most important pest of horticulture (Clarke et al., 2005; Khamis et al., 2012). It has been recovered from over 30 host plants species including cultivated and wild hosts although mango appears to be the most preferred cultivated plant (Drew et al., 2005; Ekesi et al., 2006; Mwatawala et al., 2006; Rwomushana et al., 2008). Moreover, the presence of this quarantine pest causes considerable restrictions on international trade of affected crops (Georgen et al., 2011).

The second detection of *B. dorsalis* in Mauritius was on 08 March 2013 in an orchard in the north of the island where one male *B. dorsalis* was caught in a methyl eugenol baited trap. Early detection has been possible owing to the on-going fruit fly surveillance programme since 1994. This paper summarises the methods used in eradicating *B. dorsalis* from Mauritius, the results the lessons learnt during the eradication operations. The methodology for the eradication was adopted from the Indian Ocean Region Emergency Action Plan for Exotic Fruit Flies that was developed through the regional fruit fly project entitled 'Preventing the introduction of exotic fruit fly species and implementing the control of existing species with the sterile insect technique and other suppression methods, RAF 5062' which was funded by the International Atomic Energy Agency (IAEA, 2014).

## Material and methods

The eradication of fruit flies involves an integrated approach including destruction of fallen and unwanted fruits, the application of protein bait sprays, mass trapping of males, soil drenching under selected host trees in order to kill larvae and emerging adults, fruit movement controls, release of parasitoids and release of sterile flies. In the case of the eradication programme in Mauritius, the major techniques adopted were collection and destruction of fallen fruits, fruit stripping, male annihilation technique (MAT), protein bait application technique (BAT) and soil drenching with an insecticide under selected fruit trees.

### *Early detection*

An area-wide programme for the control of fruit flies, the National Fruit Fly Control Programme, funded by the government of the Republic of Mauritius, has been operational since 1994 (Soonnoo et al., 1995; Permalloo et al., 1998; Sookar et al., 2008). This programme has, as one of its prerequisites, an island-wide monitoring system for fruit flies using male lures and food attractants (Fig.1). This island-wide trapping system made early detection of the Oriental fruit fly possible. The need for the early detection of exotic pests has been recognised as a very important factor for successful eradication (Allwood & Drew, 1997; Sewooruthun et al., 2000). The speed of detection is also a determining factor in cost, effectiveness and success of eradication.

The first detection of the Oriental fruit fly was done on the 8th of March 2013 in a locally developed methyl eugenol/malathion (ratio 1:1) baited trap based on the Steiner model. Preliminary identification of the single trapped *B. dorsalis* was done by our officers who in November 2012 followed a training course entitled 'Fruit fly surveillance, taxonomy and identification' within a Technical Cooperation Project which was funded by the International Atomic Energy Agency. Authoritative identification of the Oriental fruit fly was done by the Royal Museum for Central Africa in Belgium.

### *Delimiting survey*

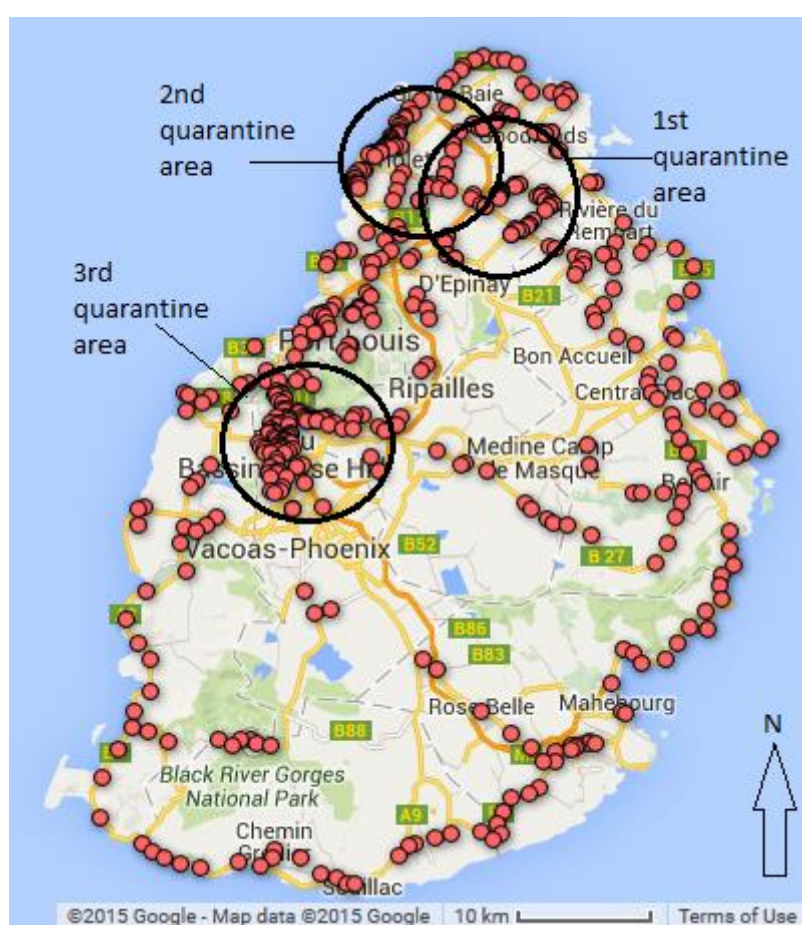
The day following the detection of *B. dorsalis* at Labourdonnais orchard in the north (Fig.1: 1st quarantine area), a delimiting survey was implemented as per the Indian Ocean Region Emergency Action Plan for Exotic Fruit Flies (IAEA 2014). The area immediately surrounding the detected fly was a core area of a 1 km x 1 km square grid. Methyl eugenol baited traps and International Pheromone McPhail trap baited with modified waste brewer's yeast were each placed at a density of 10 traps per km<sup>2</sup> within the core area. Moving outwards from the core area, there were three surrounding zones of sizes 8, 16 and 24 km<sup>2</sup>. In each of the surrounding zones, the trapping density was 2 methyl eugenol baited traps per km<sup>2</sup>. All traps were serviced weekly, with core traps serviced daily for the first week. Traps were maintained for 16 weeks after the last fruit fly find.

Four days after the first *B. dorsalis* was trapped, a second fly was detected at Trou aux Biches (Fig. 1, 2nd quarantine area) which is found at a distance of 8 km on the north-west of Labourdonnais. The delimiting survey was implemented. The last *B. dorsalis* male fly was

detected at Lablourdonnais on 21 March 2014 in a methyl eugenol baited trap (Fig. 2). On the 3rd of April 2014, three male *B. dorsalis* flies were caught in a methyl eugenol baited trap at Montagne Ory (Fig.1: 3rd quarantine area) which is around 20 km to the south-west of the first detection site. The delimiting survey was implemented. The last detection of *B. dorsalis* within the 5 km radius from Motange Ory was on 22 November 2013.

### *Fruit sampling*

Host fruit from the delimited areas were surveyed, depending on host availability. Infested fruits were collected and incubated for up to 6 weeks in pupating medium (sand) in closed, aerated plastic trays in a facility within the quarantine area. Any emerged adult was killed and preserved in alcohol or mounted for identification.



**Fig. 1.** Map of Mauritius showing trap locations (red spheres) and eradication areas.

### *Quarantine*

The eradication areas were declared quarantine by the Cabinet as per the Plant Protection Act 2006. This measure restricted the movement of fruits and vegetables outside the quarantine area. The Labourdonnais orchard was only allowed to move its fruits outside the quarantine area 16 weeks after the last detection of *B. dorsalis*.



### *Steering committee*

Immediately after the detection of the first *B. dorsalis*, a steering committee was set up under the chairmanship of the Chief Agricultural Officer for the management of actions relating to the eradication of this exotic fruit fly. Members of the steering committee included representatives of farmers, exporters of agricultural produce, the University of Mauritius, the Police, Institutions involved in agriculture and officers from the National Plant Protection Office (NPPO) and the Entomology Division of the Ministry of Agro Industry and Food Security.

### *Notification*

Notification to the international community was done by the NPPO in accordance with the requirements of the World Trade Organisation Sanitary Phytosanitary Standards Agreement, The International Plant Protection Convention and the International Standard Phytosanitary Measure (ISPM 17).

### *Publicity*

Farmers and the public in general were sensitised through the media on the detection of *B. dorsalis* and its potential threat to our agriculture. A pamphlet on *B. dorsalis* giving a list of potential hosts and measures to be taken at the home or farm level for its containment and eradication was distributed to the householders within the containment area. The measures included collection and disposal of fallen fruits and restriction on movement of fruits. Farmers and members of the public were encouraged to give information regarding damage of fruits and vegetables by fruit flies. Afterwards, fruit samples were taken for incubation in the laboratory.

### *Eradication procedures*

Eradication was initiated following the first detection of *B. dorsalis* in the delimiting survey area. For each fly detected, the area under eradication was about 25 km<sup>2</sup> surrounding the detection site. The eradication measures were pursued for eight weeks. If no fly is detected, the eradication measures were stopped and trapping was pursued for a further period of eight weeks.

#### *(a) Protein baiting*

Ground application of protein bait was carried out weekly with Hym-Lure at 10 ml/L water (Villa Crop Protection Ltd., South Africa), GF120 at 1 L/Ha (Dow AgroSciences) or modified waste brewer's yeast (WBY) at 125 ml/L water (Sookar 2001). Hym-Lure and modified WBY were mixed with Trichlorfon 80% SP (0.5 g/L water), Malathion 57 EC (2 ml/L water) or Imidacloprid 200 SL (0.5 ml/L). The protein bait was applied with knapsack sprayers and motorised sprayers mounted on the deck of trucks. Protein bait sprays were applied preferably on host trees twice a week within the core area and weekly outside the core area for a period of eight weeks from the last detection.

(b) Male annihilation technique (MAT)

The male annihilation technique involved the distribution of square (5cm x 5 cm) 1.3 cm thick, wooden plywood blocks soaked in a mixture of methyl eugenol and Malathion ULV at a ratio 3: 1 and placed at a density of 400-600 per km<sup>2</sup>, either nailed to poles or hung from trees (10 000-15 000 blocks per 25 km<sup>2</sup> fly-detection unit). A single application of MAT blocks covered a period of eight weeks.

(c) Fruit stripping and destruction of fallen fruits

As far as possible, fruit stripping and collection of fallen fruits were carried out within the quarantine areas. Stripped fruits and those collected from the ground were buried under at least 50 cm of fill. The burial site was located within the quarantine area.

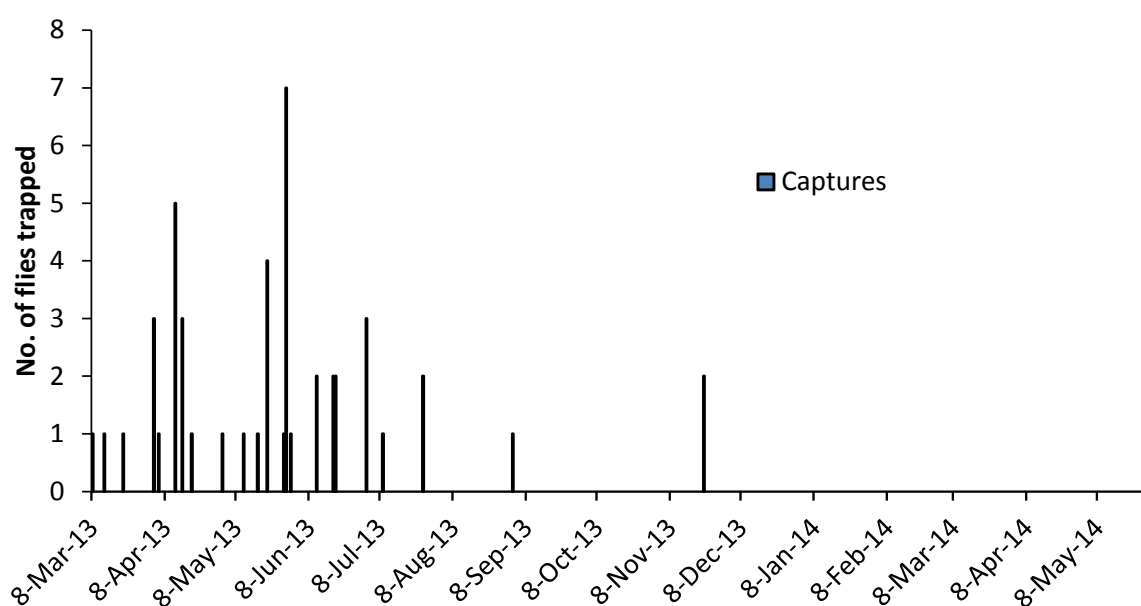
(d) Soil drenching

The soil under selected susceptible host trees was drenched with Imidacloprid 200 SL (2 ml/L).

## Results

### *Trapping of Bactrocera dorsalis*

Only one male *B. dorsalis* was trapped in a methyl eugenol/malathion baited dry trap in the 1<sup>st</sup> quarantine area compared to three males in the 2<sup>nd</sup> quarantine area during the period 08 to 21 March 2013 (Fig. 2). 39 male flies were caught in dry traps and five females were caught in wet traps baited with modified waste brewer's yeast in the 3<sup>rd</sup> quarantine area between 03 April and 22 November 2013.



**Fig. 2.** Captures of *Bactrocera dorsalis*.

### *Fruit sampling*

Table 1 (in next page) shows the fruit incubation data for the eradication areas. Fruits and vegetables belonging to 51 species were sampled for detection from the eradication area (Table 1). No *B. dorsalis* was recovered from fruits collected in the north. Emergence of *B. dorsalis* was recorded from Barbados cherry and the Indian almond which were collected in the 3<sup>rd</sup> quarantine area.

### *Materials used*

Table 2 gives a list of materials that were used during the eradication of *B. dorsalis*. Waste brewer's yeast collected from the local brewery was modified into brewer's yeast following the procedures of Lyod and Drew (1997).

**Table 2.** Materials used for the eradication of *Bactrocera dorsalis*.

Materials	Quantity
Protein hydrolysate (L)	1724
Modified waste brewer's yeast (L)	6256
GF120 (L)	38
Malathion 57 EC (L)	58
Ticlorfon (kg)	112
Imidacloprid (L)	27
Methyl Eugenol (L)	875
Malathion ULV (L)	312
EDMA traps (units)	1200

### *Fruit stripping and destruction of fallen fruits*

About 52 tons of fruits and vegetables were collected for disposal within the quarantine areas (Table 3).

**Table 1.** Fruits infestation by *Bactrocera dorsalis* (Bd), *Bactrocera zonata* (Bz), *Ceratitis rosa* (Cr), *Ceratitis capitata* (Cc), *Bactrocera cucurbitae* (Bc), *Carpomiya vesuviana* (Cv) and *Dacus ciliates* (Dc).

Fruit	Botanical name	kg	pupae/kg	Bd/kg	Bz/kg	Cr/kg	Cc/kg	Bc/kg	Cv/kg	Dc/kg
Avocado	<i>Persea americana</i> Mill.	11.55	45.59	-	27.62	0.05	-	-	-	-
Banana	<i>Musa spp.</i>	0.50	-	-	-	-	-	-	-	-
Barbados cherry	<i>Malpighia glabra</i> Millsp.	1.17	33.21	1.25	12.66	-	29.23	-	-	-
Bread fruit	<i>Artocarpus altilis</i> Parkinson	0.85	32.94	-	12.94	-	-	-	-	-
Bullock's heart	<i>Annona reticulata</i> L.	0.50	-	-	-	-	-	-	-	-
Bush passion fruit	<i>Passiflora foetida</i> L.	0.23	166.00	-	-	-	123.00	-	-	-
Butternut squash	<i>Cucurbita moschata</i> Auyama	3.90	27.04	-	-	-	-	19.26	-	-
Calabash, bottle gourd	<i>Lagenaria siceraria</i> (Molina) Standl.	0.65	-	-	-	-	-	-	-	-
Canistel, egg fruit	<i>Pouteria campechiana</i> Kunth (Baehni)	2.55	5.88	-	3.17	-	0.49	-	-	-
Ceylon olive	<i>Elaeocarpus serratus</i> L.	2.60	-	-	-	-	-	-	-	-
Chinese guava	<i>Psidium cattleianum</i> var. <i>lucidum</i> Sabine	1.39	24.79	-	-	-	15.94	-	-	-
Citrus	<i>Citrus spp.</i>	53.71	4.47	-	1.55	0.69	0.33	-	-	-
Coffee	<i>Coffea arabica</i> L.	0.03	-	-	-	-	-	-	-	-
Corinda	<i>Carissa carandas</i> L.	0.20	130.00	-	-	-	105.00	-	-	-
Cucumber	<i>Cucumis sativus</i> L.	1.35	122.50	-	-	-	-	42.50	-	-
Cucumber tree, bilimbi	<i>Averrhoa bilimbi</i> L.	2.34	10.00	-	-	-	-	-	-	-
Custard apple	<i>Annona squamosa</i> L.	10.50	11.54	-	10.14	1.39	-	-	-	-
Fig	<i>Ficus carica</i> L.	2.73	6.00	-	-	-	6.00	-	-	-

Guava	<i>Psidium guajava</i> L.	19.55	66.58	-	23.21	2.84	-	-	-	-
Hog plum, ambarella	<i>Spondias cytherea</i> Forst.	56.29	3.54	-	2.63	-	-	-	-	-
Indian Almond	<i>Terminalia catappa</i> L.	43.12	58.97	0.15	19.44	1.38	0.55	-	-	-
Indian plum, ber	<i>Ziziphus mauritiana</i> Lam.	10.45	86.50	-	35.52	-	0.21	-	1.51	-
Jackfruit	<i>Artocarpus integrifolia</i> Forst.	0.40	0.00	-	-	-	-	-	-	-
Jambolan	<i>Syzygium cumini</i> L.	1.23	0.00	-	-	-	-	-	-	-
Kumquat	<i>Fortunella</i> sp.	0.63	146.67	-	-	93.33	22.50	-	-	-
Longane	<i>Dimocarpus longan</i> Lour.	0.15	0.00	-	-	-	-	-	-	-
Loquat	<i>Eriobotrya japonica</i> F.	0.53	2.22	-	-	-	2.22	-	-	-
Malaysian rose apple	<i>Syzygium malaccense</i> Merr. and Perry	1.54	58.11	-	17.69	1.96	-	-	-	-
Mango	<i>Mangifera indica</i> L.	113.19	20.81	-	7.37	-	-	-	-	-
Marrow	<i>Cucurbita pepo</i> L.	1.10	4.17	-	13.00	1.00	-	0.83	-	0.83
Nenwa, luffa sponge gourd	<i>Luffa aegyptiaca</i> Mill.	3.00	17.00	-	-	-	-	7.00	-	-
Noni	<i>Morinda citrifolia</i> Linn.	0.40	-	-	-	-	-	-	-	-
Passion fruit	<i>Passiflora edulis</i> Sims.	5.33	4.70	-	0.46	-	-	-	-	-
Pawpaw	<i>Carica papaya</i> L.	5.65	23.70	-	-	-	-	-	-	-
Peach	<i>Prunus persica</i> L.	2.68	47.46	-	11.62	10.67	-	-	-	-
Pineapple guava (goyave de France)	<i>Feijoa sellowiana</i> O.	2.35	43.65	-	13.65	17.46	-	-	-	-
Pittaya	<i>Hylocereus undatus</i> Haw.	1.40	-	-	-	-	-	-	-	-
Pomegranate	<i>Punica grantum</i> L.	7.10	4.38	-	3.28	-	-	-	-	-
Prune, madagascar plum	<i>Flacourtia indica</i> Burm. F.	3.28	-	-	-	-	-	-	-	-

Pumpkin	<i>Cucurbita maxima</i> Duch.ex Lam.	1.00	66.00	-	-	-	-	-	-	-
Rose apple, jamrosat	<i>Syzygium jambos</i> Alston	0.10	-	-	-	-	-	-	-	-
Roselle	<i>Hibiscus sabdariffa</i> Linn.	0.30	-	-	-	-	-	-	-	-
Soursop	<i>Annona muricata</i> L.	1.15	-	-	-	-	-	-	-	-
Spanish cherry	<i>Mimusops bojeri</i> Hartog	10.55	3.73	-	0.82	-	-	-	-	-
Sponge gourd, ridge gourd	<i>Luffa acutangula</i> Linn.	0.75	89.33	-	-	-	-	81.33	-	-
Squash	<i>Cucurbita pepo</i> L.	0.98	21.54	-	-	-	-	15.38	-	-
Strawberry guava, feijoa	<i>Psidium cattleianum</i> Afzel. ex Sabine	2.93	15.21	-	4.62	0.41	2.20	-	-	-
Surinam cherry	<i>Eugenia uniflora</i> L.	0.38	48.00	-	29.33	-	-	-	-	-
Tamarind	<i>Tamarindus indica</i> L.	0.13	-	-	-	-	-	-	-	-
Tomato	<i>Lycopersicon esculentum</i> Mill.	7.70	0.44	-	-	-	-	-	-	-
Wax jambu, water apple	<i>Syzygium aqueum</i> Burm. F.	3.83	4.16	-	-	1.04	-	-	-	-



**Table 3.** Fruits and vegetables collected within the quarantine areas for disposal.

Fruits / vegetables	Botanical name	Quantity (kg)
Avocado	<i>Persea americana</i> Mill.	394
Bittergourd	<i>Momordica charantia</i> L.	30
Bottlegourd	<i>Lagenaria siceraria</i> (Molina) Standl.	29
Bread fruit	<i>Artocarpus altilis</i> Parkinson	17
Carambola	<i>Averrhoa carambola</i> L.	769
Citrus	<i>Citrus</i> spp.	20235
Cucumber	<i>Cucumis sativus</i> L.	16
Custard apple	<i>Annona squamosa</i> L.	5711
Egg fruit	<i>Pouteria campechiana</i> Kunth (Baehni)	7
Guava	<i>Psidium guajava</i> L.	16789
Hog plum	<i>Spondias cytherea</i> Forst.	809
Indian Almond	<i>Terminalia catappa</i> L.	789
Jambolan	<i>Syzygium cuminii</i> L.	1
Madagascar plum	<i>Flacourtia indica</i> Burm. F.	1
Mango	<i>Mangifera indica</i> L.	3131
Noni	<i>Morinda citrifolia</i> Linn.	113
Papaya	<i>Carica papaya</i> L.	2217
Passion fruit	<i>Passiflora edulis</i> Sims.	370
Pitaya	<i>Hylocereus undatus</i> Haw.	100
Pomegranate	<i>Punica grantum</i> L.	53
Pumpkin	<i>Cucurbita maxima</i> Duch.ex Lam	511
Ridge gourd	<i>Luffa acutangula</i> Linn.	16
Snakegourd	<i>Trichosanthes anguina</i> L.	50
Spanish cherry	<i>Mimusops bojeri</i> Hartog ex Engl.	25
Tree cucumber	<i>Averrhoa bilimbi</i> L.	4
Water apple	<i>Syzygium aqueum</i> Burm. F.	4
<b>Total</b>		<b>52191</b>

## Discussion and Conclusion

Early detection of *B. dorsalis* was possible because of the island-wide trapping system which has been in place since 1994. An emergency action plan for the containment and eradication of exotic fruit flies which was prepared under the IAEA TC Regional project RAF 5062 was available. Under the same project, the personnel were trained on the application of the fruit fly

control techniques namely application of protein bait sprays, mass trapping of males and sanitation. Preliminary identification of the trapped *B. dorsalis* was possible because the officers of the Division received training on fruit fly surveillance, taxonomy and identification. The Mauritian Government has been funding the area wide fruit fly control programme. Hence, materials and equipment were available for immediately embarking on the containment and eradication after the first detected *B. dorsalis*. The steering committee monitored the progress of the eradication programme. The last detection of *B. dorsalis* in trap dates back to 22 November 2013. This fly was recovered from collected Barbados cherry and the Indian almond only.

We should improve our quarantine capacity in order to prevent any introduction of exotic fruit flies. The use of X-Ray machines for the detection of illegal entry plant materials in luggage and hand bags at the ports of entry is being contemplated. In order to reduce the risk of accidental entry of exotic fruit flies, imports of fruits and vegetables are allowed from pest free areas or after proper quarantine treatment. The fruit fly surveillance system should be reinforced with traps placed in a grid so as to detect incursion of pest species as early as possible.

### Acknowledgements

The *B. dorsalis* eradication programme was funded by the Government of Mauritius. The IAEA supported the campaign by providing training on fruit fly surveillance and management to the staff of the Entomology Division. The local brewery provided free waste brewery yeast for use as fruit fly bait.

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## Popularizing IPM of fruit flies in cucurbits and subtropical fruits through an area wide approach in north western Himalaya

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### Abstract

Himachal Pradesh is a hilly state located in north western Himalayan region of India, where variety of fruits and vegetables are being cultivated. Among insect pests, fruit flies (Diptera: Tephritidae) in particular are the major hurdle for the successful and profitable cultivation of fruits and vegetables even after continuous use of broad spectrum insecticides. Fruit flies namely *Bactrocera cucurbitae*, *B. scutellaris*, *B. tau*, *B. dorsalis* and *B. zonata* cause huge economic losses to the tune of 35 -80% in different crops. The existing recommendations fail to target the adults, eggs and the developing maggots, besides frequent insecticidal applications leaving harmful residues on fruits and vegetables making them unsuitable for human consumption. To save huge damage to the crops, an area wide management of fruit flies was started based on mass trapping of male and bait application to the crops in this region. A low cost bottle trap (pheromone traps) based on prevalent fruit fly species was developed with cue lure and methyl eugenol and validated during 2009-2010 along with other pest management modules viz. sanitation by destroying fallen fruits and application of bait splash (gur/ jaggery mixed with a mild toxicant) on farm trials. The growers were educated and motivated through awareness campaigns, trainings and demonstrations on the economic and environmental importance and value of fruit fly management using male annihilation method during the years 2010-2013. The fruit fly management module is being demonstrated with the active participation of farmers and assistance from other developmental departments especially Department of Agriculture, Himachal Pradesh since 2010. The fruit fly trapping and management technology has been popularized in almost all the fruit fly infested districts of the state and now being adopted in adjoining states. Fruit fly traps installed @ 25 traps/ ha along with bait application and sanitation not only resulted in about 40-60 percent reduction in fruit fly population but also increased yields by 20-30 percent and reduced number (reduction in 5 sprays) of insecticidal applications saving labour costs, time and environment. Owing to area wide adoption of the technology, it horizontally spread in about 1000 ha in the state till now and more than 50000 traps have been supplied to the farmers, in addition to neighbouring districts of Haryana. The cost benefit ratio of IPM module was 2.54-2.81. An area wide fruit fly trapping along with other environment friendly technologies with participation of different stakeholders and person to person contact/ education is the way out to achieve success in elimination of fruit fly complex and minimize the economic damage.

**Keywords:** area wide management, cucurbits, fruit fly suppression, mass trapping, pheromone traps.

## Introduction

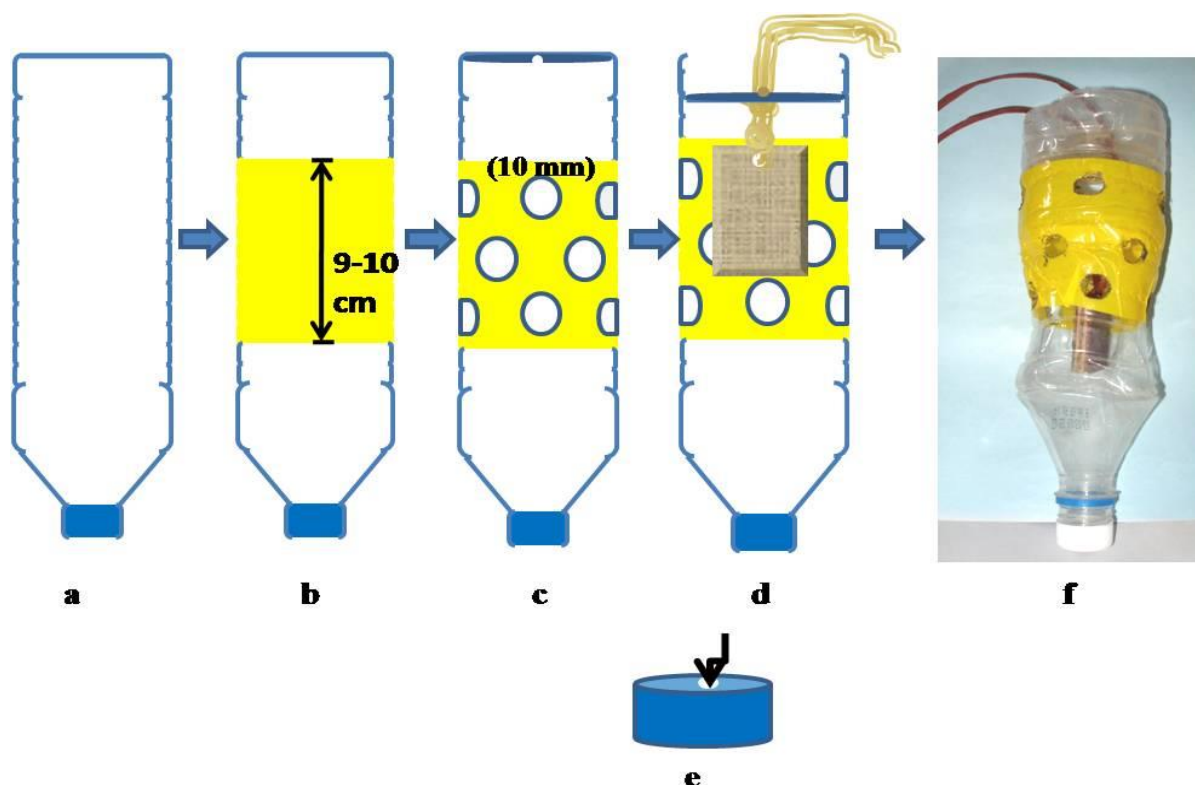
Cucurbits mainly comprise of cucumber, bottle gourd and bitter gourd, which are the important crops grown in Himachal Pradesh in an area of 2443 ha with production of 54237 tonnes (Anonymous, 2010). Tephritid fruit flies (Diptera: Tephritidae) are one of the most diverse group of insects, and cause annual economic loss of about Rs 6,958.20 crore in India (Sardana et al., 2005). Fruit flies in the subfamily, Dacinae occur throughout the tropics and subtropics (Drew, 1992) and are signified as one of the most destructive pests of fruits and vegetables. Females lay eggs inside the host and developing maggots grow and causes rotting of the plant tissues. The early detection of infestation is very difficult which often lead to rotting of fruits. The peak pest population coincides with the onset of monsoon rains, hence a major proportion of applied pesticides get washed, necessitating repeated applications, which however pose serious health hazards to the consumers.

The melon fly, *B. cucurbitae* (Coquillett) and pumpkin fly, *B. tau* (Walker) have wide distribution throughout South-East Asia and attack various crops (White & Elson-Harris, 1992; Huque, 2006). A number of pest management tactics for *B. cucurbitae* like change in sowing seasons and selection of crop varieties (Borah & Dutta, 1996; Joshi et al., 1995), use of trap crops (Khan & Manzoor, 1992) and cultivation practices to destroy diapausing pupae in the soil (Agarwal & Sueyoshi, 2005) have been advocated. During the past six decades however, the efforts to manage the insect-pests were dominated by chemical preparations which have led to environmental pollution, destruction of natural enemy complex and development of resistance against toxicants in over 500 species of insects and mite pests apart from undesirable side effects to human health and destabilization of agro ecosystems. So there is an urgent need to look for safer eco-friendly alternatives in view of pressing need for sustainable development in agriculture sector. Semio chemical (especially pheromone) based pest management technologies are fast emerging as effective and possible alternates in the hilly and isolated ecosystems. The effective management of pest could only be possible through mass trapping of the male fruit flies (male annihilation technique- MAT) followed by need based bait application technique (BAT). Efforts were made to develop an innovative mean to manage this hazardous pest which could be befitted well under both conventional as well as organic farming situations. Para pheromone based Palam trap is a non-chemical, safer and eco-friendly mean for fruit fly management based on behaviour, ecology and para pheromone based male annihilation technique. The present studies were henceforth conducted to evaluate the effectiveness of the trap as an integral part of IPM module over the locations and to standardize and disseminate the management strategies as an area wide approach in collaboration with various stake holders against fruit flies.

## Material and Methods

To study the extent of losses caused by the pest in cucurbits (bitter gourd and cucumber), experiment was conducted on farmer's field in three districts (Mandi, Kangra & Hamirpur) during crop seasons of 2010 and 2011. The experimental plot was divided into two sets i.e. protected and unprotected. In protected plots, the crop was protected from fruit fly damage by following regular application of gur + malathion (5-6 applications) at weekly intervals, while no insecticidal application was made in case of unprotected plots. Percent fruit damage due to fruit flies were worked out in both protected and unprotected plots at each picking, The data was used to work out percent avoidable losses due to the pest.

Palam traps (Fig.1) were installed @ 4 traps/ ha at ten different locations in Mandi district and weekly catch of fruit flies data were recorded. The fruit flies trapped were identified and species composition of fruit flies was worked out. The identification of fruit fly species infesting cucurbits was done on the basis of morphological descriptions given by White & Elson-Harris (1992) and Drew & Raghu (2002).



**Fig. 1.** Structural design of Palam trap; a: Remove the wrapper of bottle if any; b: cover the middle of the bottle (9-10 cm) with yellow adhesive tape; c: insert holes (10 mm) in the middle area; d: fitting of wooden block lure septa into bottle; e: a small hole in the bottle cap for rain water drainage; f: ready to install Palam trap in to the field.

On farm trials for the management of fruit flies involving three treatments viz. fruit fly traps only @ 25/ hectare, fruit fly traps+ BAT (50 g gur+ 10 ml malathion in 5 litre of water thrice



at 15 days interval) and control (50 g gur+ 10 ml malathion in 5 litre of water thrice at 15 days interval, old package of practices) at ten locations in the district were conducted. The traps were installed at the initiation of flowering in both the crops. Percent fruit damage and yield data was recorded in all the treatments to work out the economics of the treatments. The data recorded for different parameters in the present study were analysed statistically and the treatment means were compared at 5% level of significance by least significant difference test described by Gomez and Gomez (1984).

Based on the results of the trials, the management strategies were included in the package of practices of the university and area wide approach with the involvement of various stake holders, especially department of agriculture, Government of Himachal Pradesh was adopted for large scale dissemination of technology over the locations in the state.

## Results and Discussion

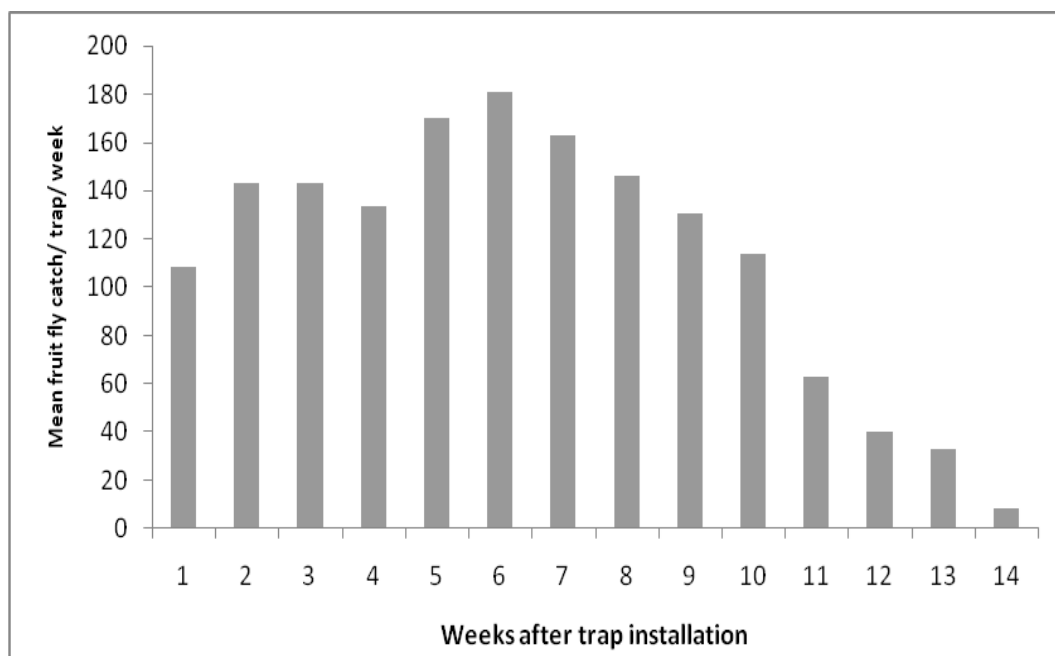
The studies revealed that the avoidable losses due to fruit flies varied from 89.7 to 94.6 percent at different locations of the state. In Hamirpur district, the avoidable losses were maximum, followed closely by Mandi and Kangra (Table 1). High fruit fly infestation in cucurbits recorded during the present study in Himachal Pradesh is in accordance with Gupta et al. (1992) and Sood et al. (2010) who had observed infestation to the tune of 60-80 percent due to fruit flies on different cucurbits in Himachal Pradesh.

**Table 1.** Avoidable losses due to fruit flies in cucurbits.

Locations	Percent fruit damage			Avoidable losses (%)
	Protected*	Unprotected	Mean	
Palampur (32°6' N, 76°32' E)	37.2	69.1	53.2	89.7
Mandi (31°31' N, 76°54' E)	29.7	58.3	44.0	91.5
Hamirpur (31°46' N, 76°20' E)	30.8	64.0	47.4	94.6

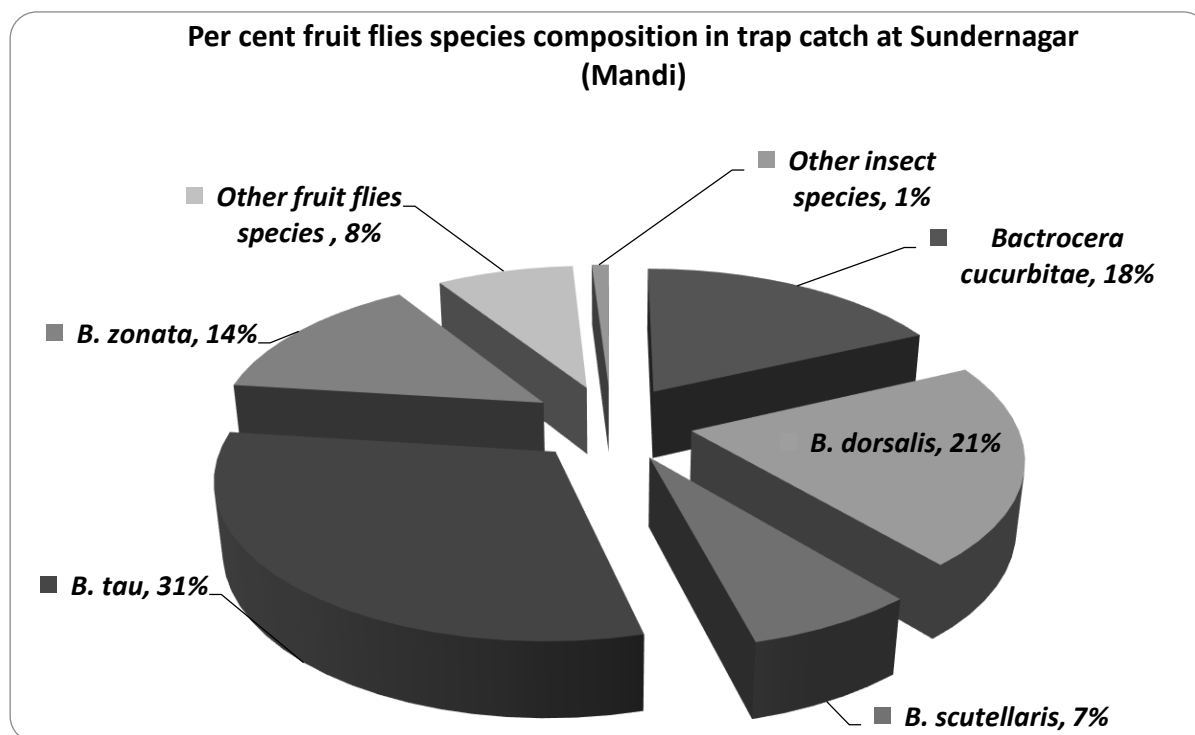
\* Protected: Three applications of gur + Malathion

The mean trap catch per week in Palam trap recorded at Sundernagar was 112.6 and the traps were found quite effective for up to 12th week of installation after which the trap catch reduced drastically (Fig. 2) and the traps were recharged preferably after 12 weeks of installation. Though, there was less direct impact on reduction of fruit damage as observed under field experiments, however when installed on a mass scale the traps were found to have a great impact on reduction of fruit fly incidence and pesticide usage.



**Fig. 2.** Weekly trap catch data of fruit flies at Sundernagar (Mandi).

*Bactrocera tau*, *B. dorsalis* and *B. cucurbitae* were the predominant fruit fly species at majority locations surveyed in Mandi (Himachal Pradesh). Of the total fruit flies trapped, *B. tau* comprised of 31 percent (Fig. 3), followed by *B. dorsalis* (21 %), *B. cucurbitae* (18 %), *B. zonata* (14 %), *B. scutellaris* (7 %) and other fruit flies (8 %). *B. cucurbitae* was earlier considered to be the major fruit fly species infesting cucurbits in Himachal Pradesh, however during the course of present survey, *B. tau* and *B. cucurbitae* both were recorded as the predominant species infesting cucurbits in Himachal Pradesh. These species have earlier been reported to be the major species infesting cucurbits (Kapoor et al., 1980 and Gupta et al., 1992) in Himachal Pradesh. However, Sood and Nath (1999) and Prabhakar et al. (2009) reported *B. tau* as a major fruit fly species infesting cucurbits in the State. *B. tau* has also been reported from north-eastern region of India (Borah & Dutta, 1996), China (Yang et al., 1994) and Bangladesh (Huque, 2006). The reports pertaining to cucurbit infestation by *B. tau* from across the places indicate a wider geographical distribution of the species. During recent past, *B. scutellaris* has also been reported as one of the most destructive fruit fly species infesting tender fruits and growing vegetative parts from Himachal Pradesh, damaging not only the fruits but also retarding the plant vigour and growth (Prabhakar et al., 2007; Sunandita & Gupta, 2007; Prabhakar et al., 2009). The reports of different workers on infestation of cucurbits by fruit flies in the Himachal Pradesh substantially support the present findings, that cucurbits are not damaged by a single fruit fly species but by a complex of species viz. *B. cucurbitae*, *B. tau* and *B. scutellaris*.



**Fig. 3.** Species composition of fruit flies at Sundernagar (Mandi).

The impact of trapping was more visible in subsequent years of trapping. Till now, the recommendation for pest management was only chemical based (bait application only) and non-availability of recommended chemicals like fenitrothion and fenitrothion is compelling the farmers to use other insecticides, hence inclusion of trapping as a component of fruit fly management not only ensured the protection of the crop but also reduced frequent insecticide applications, which was posing serious environmental and health hazards. The technology of male annihilation using Palam fruit fly traps @ 25 traps/ ha along with the BAT (gur + malathion application) has been included in the package of practices instead of cover spray of 50 g gur+ 10 ml malathion in 5 litre (old package) of the university. Effective management of fruit flies with an area wide management approach at village level has already been demonstrated at many places in India (Stonehouse et al., 2007). This technology has reduced the fruit fly damage and enhanced the effectiveness of bait application, owing to which trapping has been made an important component of fruit fly management by the state department of agriculture for area wide adoption in all the districts. More than fifty thousand traps have been supplied to the farmers during the last five years for area wide management of pest in all the fruit fly infested areas of the state and the fruit fly trap is becoming popular amongst the farmers as an integral part of wide area fruit fly eradication programme. The successful intervention is also being percolating across the state boundaries as the technology has been demonstrated in some adjoining districts of Haryana and has been found equally effective. Area wide fruit fly trapping along with other environment friendly technologies with participation of different stakeholders and person to person contact/ education is further encouraged to achieve complete success in elimination of fruit fly complex and minimize the economic damage.

**Table 2.** Effect of fruit fly traps on fruit damage and cucurbits yield in Sundernagar (Mandi).

Treatments	% fruit damage*		Yield (q/ha)*			Gross Returns		Cost of cultivation		Benefit cost ratio	
	Bottle guard	Cucumber	Bottle guard	Cucumber	Mean	Bottle guard	Cucumber	Bottle guard	Cucumber	Bottle guard	Cucumber
Traps only @ 25/ hectare	55.03	58.60	98.8	89.2	93.5	98800	89200	50400	53100	1.96	1.68
Traps+ BAT (50 g gur+ 10 ml malathion in 5 litre of water thrice at 15 days interval)	28.20	30.15	146.0	138.7	142	146000	138700	51900	54600	2.81	2.54
Control (50 g gur+ 10 ml malathion in 5 litre of water thrice at 15 days interval)	39.74	41.47	121.1	114.6	117.5	121000	114600	46900	49600	2.57	2.31
LSD (5%)	9.08	10.27	15.2	16.9							

\*Average of ten locations; \*\* Trap cost @ Rs. 100 per trap and installed twice in the season; \*\*\* Produce cost- Rs. 1000 per q

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## Socio-economic analyses of area-wide management of mango fruit fly in South India

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### Abstract

**Background:** Mango is the most economically important fruit crop of India. The most important mango belt in Karnataka is Srinivasapura taluk (subdistrict) of Kolar district and it is the main crop of the taluk and hence the economy is dependent on it. One of the major limitations to mango productivity is the loss due to Oriental fruit fly (*Bactrocera dorsalis*) infestation. So the objective of the present study was to demonstrate the Integrated Pest Management (IPM) technology for fruit flies to mango farmers and to study the social impacts, constraints, economic improvements from adoption and the acceptability and potential uptake of the technology by farmers.

**Methods:** Fruit fly methyl eugenol traps developed at the Institute were placed in selected orchards with the help of local horticultural officers. Fruit flies trapped were brought to the laboratory, labelled and processed for taxonomic examination. The study was carried out with the help of the Karnataka State Horticulture Department and the Mango Growers Federation of Srinivasapura. In the mango seasons of 2007 to 2009 fruit fly infestation levels were monitored by field surveys. Mature fruits (n=200 to 400 fruits of assorted cultivars) were sampled at random from 2-3 different orchards/per village. At least eight villages spread across the taluk at one to seven days prior to harvest were sampled. Percent infestation was calculated and adult flies were labelled for taxonomic processing. The fruits examined were mainly from cultivars Totapuri, Alphonso, Banganpalli and Neelum. The Integrated Pest Management consisted of male annihilation using methyl eugenol traps (15-20 traps/ha), destroying fallen fruits and bait splashes with jaggery (10%) +dichlorvos 78 EC (toxicant 2ml/l of bait) on the base of the main trunk, approximately 30cm above the ground @ 50ml bait/ tree.

**Results:** Surveys conducted from 2007 to 2009 showed that mean infestation of fruit fly was 48.3% on assorted cultivars of mango grown in Srinivasapura. Productivity enhancement was achieved to an extent of 45.8% in the 62.3ha of demonstration area. This clearly indicated that a fruit lost to fruit fly, if saved, is a fruit gained. The impact of this intervention was found to give a mean yield increase of 124.53 tonnes across the 62.3ha. This resulted in a productivity increase from 4.37 tonnes to 6.37 tonnes/ha, an increase of 1998.9 kgs of fruit/ha.

**Conclusions:** Economic analysis in 2012 showed that the technology spread to at least 55% of the mango area across south India with a net benefit of Rs. 5156 crores (approximately



US\$825mn at 2014 exchange rate) of increase in mango revenue. The impact analysis showed that these farmers realized at least 20-40% yield increase, with a cost:benefit ratio ranging from 1:4 to 1:57, depending on the commercial value of the mango variety.

*Keywords:* *Bactrocera dorsalis*, integrated pest management, area-wide, mango

## Introduction

Mango is the most important fruit crop of India. In south India, Karnataka is the second most important mango growing state after Andhra Pradesh. The most important mango belt in Karnataka is Srinivasapura taluk (subdistrict) of Kolar district. The main commercial varieties grown there are Totapuri, Neelam, Banganapalli and Alphonso. In addition, the area has other varieties such as Himayun Pasand, Raspuri, Kalapad, Mallika, Amarapalli, etc. Srinivasapura is a locally important market hub for mango, where nearly 80,000 tonnes of mango are marketed from makeshift packhouses. During the season, several middlemen and traders with temporary possession of orchards (through auction) also operate here. Mango is the main crop of the taluk and hence a major portion of the local economy is dependent on it. The taluk is one of the main feeders of fresh fruits to major urban markets of India such as Bengaluru, Mumbai, Chennai, Delhi, Kolkata, etc, besides the Gulf countries, Singapore and Malaysia. It also supplies (especially the variety Totapuri) to processing industries in Chittoor (Andhra Pradesh), Krishnagiri (Tamil Nadu), etc. Therefore, maximizing mango productivity is crucial to the economy of Srinivasapura.

One of the major limitations to mango productivity is the loss due to Tephritid fruit fly (Diptera: Tephritidae) infestation, mainly due to *Bactrocera dorsalis* (Hendel) species. The average national loss of mango due to fruit flies is estimated to be 27% (Verghese et al., 2006), with higher losses in the southern mango belts and lower losses in the northern belts. It was felt that if the mango farmers in Srinivasapura adopt the Integrated Pest Management (IPM) technology, developed by the Institute through an ICAR-DFID (UK) collaborative programme between 2001-2005 (Stonehouse et al., 2005) and further refined by the Division of Entomology and Agriculture Technology Information Centre, on an area-wide basis, the loss of mature fruits due to fruit flies could be substantially reduced, thus enhancing yield. So the objective of the present study was to demonstrate the IPM technology of fruit flies to mango farmers and to study the social impacts, constraints and economic improvements from adoption and the acceptability and potential uptake of the technology by the farmers.

## Material and Methods

In Karnataka, Kolar district has the highest mango area with 40,769 ha, and so this district was chosen for transfer of technology (TOT) related to area-wide integrated pest management (awIPM) of fruit fly. Srinivasapura taluk of Kolar has more than 21,125 ha, of mango. So, this taluk was the focal point for the TOT of awIPM. Fruit fly parapheromone traps developed at

the Institute were placed in selected orchards with the help of local horticultural officers. Fruit flies trapped were brought to the laboratory, labelled and processed for taxonomic examination.

The study was carried out with the help of the Karnataka State Horticulture Department and the Mango Growers Federation of Srinivasapura. In the mango seasons of 2007 to 2009, fruit fly infestation levels were monitored by field surveys. Mature fruits (n=200 to 400 fruits of assorted varieties) were sampled at random from 2-3 different orchards/per village. At least eight villages spread across the taluk at one to seven days prior to harvest were sampled. These fruits were cut and examined in the field and percent infestation was assessed. Harvested fruits were also sampled from the market yard, with the help of traders. Sampled fruits were bought (at wholesale rate) and brought to the laboratory for ripening and observation of infestation (n=3-10 kgs). Percent infestation was calculated and adult flies that eclosed were killed with ethyl acetate vapour in the post-teneral phase and labelled for taxonomic processing. The fruits examined were mainly Totapuri, Alphonso, Banganpalli and Neelum varieties. The Integrated Pest Management consisted of male annihilation using methyl eugenol traps (15-20 traps/ha), destroying fallen fruits and bait splashes with jaggery (10%) + dichlorvos 78 EC (toxicant 2ml/l of bait) on the base of the main trunk, approximately 30cm above the ground @ 50ml bait/ tree.

#### *Technology dissemination*

The awIPM consisted of placing parapheromone traps @ 15-20/ha in mango orchards at preharvest stage (from approximately 30-45 days prior to harvest). Weekly collection and destruction of fallen fruit during the same period was advocated, followed by application of the bait described above on tree trunks twice before harvest at one week interval. The technology had no components of insecticidal spray either on the tree canopy or fruits and was therefore relatively environment-friendly and completely residue-free on the fruit. These measures were recommended to be implemented on wide-area, preferably in contiguous belts. There were several ways by which the TOT took place through extension folders, farmer campaigns, field demonstration, radio/television programmes, etc.

## **Results and Discussion**

### *Infestation and species of fruit fly*

Surveys conducted from 2007 to 2009, prior to the IPM introduction, showed that mean infestation of fruit fly was 48.27% on assorted varieties of mango grown in Srinivasapura, higher than the national average levels. The flies that emerged from fresh fruits were all identified as *Bactrocera dorsalis*, based on the key developed by Madhura and Verghese (2004). Despite three fruit fly species were identified in the traps (Table 1) only *B. dorsalis* was found infesting mango.

**Table 1.** Total of different fruit fly species of Srinivasapura caught in parapheromone traps (n=2 traps, village).

	<i>B. dorsalis</i>	<i>B. zonata</i>	<i>B. correcta</i>	Remarks
2007	25	0	0	Site A
2008	329	2	22	Site A
2009	80	6	8	Site B

The economy of the Srinivasapura taluk (Kolar district of Karnataka, South India) is largely dependent on mango. Productivity enhancement was achieved to an extent of 45.8% in the 62.3 ha of demonstration area. During seminars, the farmers were made aware of the species of fruit fly infesting mango and the nature of damage, biology of the pest, the loss being caused in the area and details of the intervention required on an area-wide basis. Each farmer was asked to take up this intervention on at least 0.80 ha for which 12 free traps were to be given to them. It was felt that farmers may hesitate to invest in the traps at the initial awareness stage, but if the farmers personally experience the benefit of IPM, they would adopt it in subsequent years. It is estimated that there are around 10,500 mango farmers in Srinivasapura taluk. In and around Srinivasapura town, there are approximately 500 mango farmers who might potentially respond to invitations for free distribution of traps. The publicity through two local newspapers and Department of Horticulture elicited a response from only 116 farmers, even though the traps were free. All these farmers were ones who managed their mango orchard without auctioning to a trader. This also gave an indication that those farmers who had auctioned their orchards prior to harvest were not interested in the IPM using fruit fly traps. According to the President of the local mango growers association, 50% of farmers in the area were self-marketing farmers, so the group of 116 farmers out of 250 self-marketing farmers accounted for 46.40% of the likely uptake group.

The seriousness of the farmers who took the traps, for the adoption of IPM, can be gauged from the fact that only 32% of the farmers placed the traps within the first week of obtaining them. Another 34% placed the traps the following week, thus in the first fortnight only 66% of the farmers installed the traps. This was approximately a month prior to harvest and hence the right time of intervention. Thus an adoption of 66% was obtained for the technology transfer, while the remaining 34% either did not place the traps or installed them too late to get any effective control. The 66% constituted 77 farmers. This accounted for 30.50% of the self-marketing target farmers in the area. The overall percentage of farmers who adopted the IPM was 15.2%. The traders, who auctioned or took farms by lease, did not show interest in collecting and implementing the traps. However this group will stand to immensely benefit if IPM is adopted, as fruit flies affect the mango crop directly. A fruit saved means extra income to the trader. As each farmer installed the trap in 0.80 ha, the total area covered was 62.3 ha in the selected area of Srinivasapura taluk. All these 77 farmers were interviewed for their satisfaction with the AWIPM intervention. It was found that the level of satisfaction

expressed by the farmers was positively correlated to the level of fruit fly catch. All the 77 farmers were interviewed for their perceived yield increase by mitigating loss due to fruit flies. For smaller land holdings the perception of yield increase was higher, while farmers with a higher land holding perceived less yield increase. One main reason which could be attributed to this is that small farm holders are invariably present in and around the orchards and are able to assess IPM interventions more accurately than larger farm holdings, where absentee landlords are the norm. The mean loss due to fruit flies estimated in the three years prior to the transfer of technology was 48.27% and the mean yield increases perceived was 45.8%. This clearly indicated that a fruit lost to fruit fly, if saved, is a fruit gained.

The estimated impact of this intervention in the 62.3 ha of IPM implementation was a yield increase of 124.53 tonnes, compared to farmer reports of recent previous harvests. This resulted in a productivity increase from 4.37 tonnes to 6.37 tonnes/ha, which is an increase of 1998.9 kgs of fruit/ha. If the fruit is sold at an average cost of Rs 20/kg (minimum prevailing market rate across commercial varieties) the farmer gains Rs. 39,978/ha which works out to a benefit of approximately Rs 57 for every rupee spent on IPM. The cost of IPM was approximately Rs. 700 for every hectare. This huge benefit served as an ideal demonstration to local farmers and hopefully more farmers would adopt the technology. Then in this context, it should also be mentioned that the technology should be also adopted by non-farming traders who temporarily take the orchards on lease through auction as they can also realize more yield and hence income, as they constitute 50% of the market. It is hoped that they would also adopt the technology.

Economic analysis in 2012 showed that the technology had subsequently spread to at least 55% of the national mango area with a net benefit of Rs. 5156 crores (approximately US\$825mn at 2014 exchange rates) of increase in mango revenue. FAOStat figures for 2012 estimate total mango production in India at around 15mn tonnes, worth approximately US\$ 5bn at farm-gate prices. The impact analysis showed that these farmers adopting IPM realized at least 20-40% yield increase, with a cost:benefit ratio ranging from 1:4 to 1:57, depending on the commercial value of the mango variety. All the farmers expressed high satisfaction with the area-wide IPM of fruit flies. Furthermore, the loss saved resulted in increased business in the mango pulp industry with gainful employment of women especially in rural areas.

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## Area-wide suppression of *Bactrocera* fruit flies in dragon fruit orchards in Binh Thuan, Viet Nam

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### Abstract

**Background:** Fruit flies, namely *Bactrocera dorsalis* and *Bactrocera correcta*, cause extensive losses to many kinds of fruits in Viet Nam, including two species of dragon fruit (*Hylocereus undatus* and *Hylocereus polyrhizus*, white and red flesh respectively), which have a high economic value for export. Total planted area of dragon fruit in Viet Nam is estimated to be approximately 30,000 ha at present, with about 22,500 ha cultivated in Binh Thuan province alone. Insecticide application used on a farm-by-farm basis has been found to be inefficient to control fruit flies on dragon fruit and could lead to development of insect resistance and increase of insecticide residues on fruits, on top of negative impacts on humans and environment. A pilot fruit fly control programme was initiated in 2013 with comprehensive objectives including: 1) to suppress fly populations on dragon fruit fields and 2) to assess economic benefits that can be achieved through the implementation of an area-wide integrated pest management (AW-IPM) programme.

**Methods:** The area for the pilot test consisted of a 100 ha core zone (1 km by 1 km) for testing the effectiveness of IPM programme surrounded by a 300 ha buffer zone (2 km by 2 km). The dragon fruit area outside the buffer zone used existing farmer's practices (farmer's practice zone) and served as a control. The experiment was started in July 2013. Three suppression methods applied in the core zone consisted of (1) Field sanitation, including alternate host removal and stripping and destruction of unwanted dragon fruits, (2) Male annihilation technique (MAT) using methyl eugenol + fipronil blocks placed at 50 m intervals across the field, and (3) a mixture of beer waste protein baits with fipronil applied weekly to the leaves during the dry season or placed in bait bottles during the rainy season. In the buffer zone, both sanitation and MAT methods were used. Population monitoring was conducted in the core, buffer and farmer's practice zones using a grid of traps baited with methyl eugenol. The traps were placed 1 day after the experiment started, inspected every 7 days and serviced every 6 weeks. These traps provided spatial and temporal data on fly incidence in all 3 zones. In addition to dragon fruit, seven other host fruits i.e. rambutan, rose apple, custard apple, guava, Barbados cherry and mango were sampled for fly infestation to further evaluate the effectiveness of the suppression measures.

**Results:** Data collected from August 2013 to July 2014 found that the populations of *B. dorsalis* and *B. correcta* decreased in both the core and buffer zones (a reduction of 3.5 to 0.7

and 3.0 to 0.9 flies per trap per day, respectively), whereas there was an increase in the number of flies trapped in farmer's practice zone (2.6 to 3.6 flies per trap per day). The average percentage fruit damage over most of the secondary season (October 2013 to - February 2014) in the core, buffer and farmer's practice zones was 2.9, 3.5 and 5.9%, respectively. In part of the main fruit season (June to September 2014), the average percentage fruit damage was 2.5% in core zone, 5.7% in buffer zone and 9.9% in farmer's practice zone.

*Conclusions:* Area-wide suppression methods using MAT, spraying protein bait spray alone or in combination with sanitation, including alternate host removal and stripping and destruction of unwanted dragon fruit, was found to be effective for controlling fruit fly populations in dragon fruit farms in Binh Thuan province. Creation of areas of low pest prevalence using area-wide suppression would decrease pesticide use, improve dragon fruit value, and expand the dragon fruit export market.

*Keywords:* fruit sampling, male annihilation technique, protein bait, sanitation, trapping.

## Introduction

Binh Thuan is a province in the south central coast of Viet Nam that has favourable weather and land conditions for growing dragon fruit. At present, the area under dragon fruit cultivation is 22,500 ha, with 30,000 farmer households, producing about 400,000 tons per year, of which 80% is exported to over 14 countries including China, Japan, Korea, Taiwan, USA and some countries in Europe. One major limitation is infestation by fruit flies that subject dragon fruit to strict quarantine measures and limit the export market.

Currently cover sprays of insecticides are commonly used by the dragon fruit industry to prevent and eliminate fruit flies. This practice is undesirable because it may lead to the development of insecticide resistance, it may result in an increase of insecticide residues in the fruits, and also has negative human and environmental impacts. About 35% of the dragon fruit growing area follows the Vietnamese Good Agricultural Practices (VietGap) and Good Agricultural Practices (GlobalGAP) standards, which include farmer use of the male annihilation technique (MAT). However, this method for suppressing fruit fly populations is not very effective in Viet Nam overall as well as for dragon fruit in Binh Thuan province. Therefore, a comprehensive control method in an area wide is needed.

To assist the dragon fruit industry tackle the fruit fly problem the Plant Protection Research Institute initiated an area-wide fruit fly demonstration suppression project over 400 ha of dragon fruit orchards in Binh Thuan province in July 2013. The project was implemented with the support of the Joint Food and Agriculture Organization of the United Nations and International Atomic Energy Agency (FAO/IAEA) Programme through an IAEA Technical Cooperation Project: "VIE 5017: Supporting Area-Wide Integrated Pest Management to Improve the Quality of Fruit for Export". The study also included an infestation survey of

alternate host fruit when present. The results of this area-wide fruit fly suppression project are reported here.

## Material and Methods

### *Survey on host range of fruit fly in Binh Thuan province*

The survey involved common fruits that are potential fruit fly hosts and were also present in the dragon fruit production area. Samples of these fruits were collected at random and placed into plastic bags, labeled and transported to the laboratory. Approximately five kilos of each fruit species or variety were sampled. The fruits were examined individually and assigned into one of the following 4 groups: early damage, medium damage, late damage, and no damage symptom. The fruits were then kept individually in plastic containers and monitored further for the presence of larvae and pupae, if any. Pupae were then collected and kept in boxes containing 5 mm layer of sawdust and provided with moisture for adult emergence. Emerged adults were fed with a mixture of sugar and protein and reared for about 5 to 7 days before being killed by freezing and identified to the species level.

### *Area-wide of suppression fruit flies*

The demonstration project was conducted at Ham Hiep commune (400 ha), Ham Thuan Nam district, Binh Thuan province. This area contained a high concentration of dragon fruit farms. Environmental conditions in the Ham Thuan Nam district include average temperature of 26-27°C, average humidity of 78-85%, and total average annual rainfall of 800-2000 mm. The rainy season is from May to October and the dry season from November to April. The main dragon fruit season is from April to October. Outside of the main season, farmers use electric lights to stimulate flowering for a second season, from November to March. Thus, dragon fruit is available and subject to fruit fly infestation all year round.

The area-wide fruit fly suppression project consisted of a 100 ha core zone (1 km by 1 km) surrounded on all sides by a 300 ha buffer zone (2 km by 2 km). The area surrounding the buffer zone on all sides consisted of more dragon fruit farms and was used as a control or farmer's practice zone.

Three suppression methods were utilized, namely:

- (i) Sanitation, including alternative host removal, stripping and destruction of unwanted dragon fruit;
- (ii) Male annihilation technique (MAT): Medium density fibreboard about 1 cm thick was cut into 5 by 5 cm blocks. A small hole was drilled in the corner of each of these wooden blocks for a nylon wire to pass through. Four ml of fipronil was mixed into 1 litre of methyl eugenol and the wooden blocks were soaked in this mixture for 4 days. The prepared wooden blocks were then hung at 50 m intervals in a grid and the blocks were replaced every 10 weeks and maintained continuously throughout the year in the field.



(iii) Bait application technique (BAT): Protein bait was prepared by mixing 1 litre of Ento-Pro 150 DD in 9 litres of water and adding 1 g of fipronil 800 WG. The bait was applied as spot spray on leaves of host trees (50 ml/tree) and on dragon fruit plants weekly at a rate of 10 litres/ha. Application of protein bait was also conducted from mid-April to mid-June on alternate hosts such as mango. However, in the rainy season (from May to October) protein bait was contained in plastic bottle traps hung in the host trees. To prepare these bait stations, 3 parts Ento-Pro 150 DD were mixed in 7 parts water and 1 g of fipronil 800 WG. Each bait station contained of 200 ml of protein bait + insecticide solution. Twenty five bait stations were used per hectare.

All three treatments, i.e. sanitation, MAT and BAT, were applied in the core zone. In the buffer zone, only sanitation and MAT were utilized. In the farmer's practice zone, usually used about three methyl eugenol traps per hectare, mainly to monitor adult fly populations. They then applied cover sprays of insecticides when they detected adult flies in the traps. However, not all households practised this and some growers did not apply any control methods at all.

#### *Evaluation of the fruit fly suppression*

Populations of the males of both *B. dorsalis* and *B. correcta* in the core, buffer and farmer's practice (control) zones were monitored using modified Steiner type traps baited with methyl eugenol. The number of traps used were 11 traps (1 trap per 9 ha) in the core zone, 21 traps (1 trap per 14 ha) in the buffer zone and 8 traps (set equidistant and surrounding the buffer zone on all sides). Traps were serviced every 7 days and the numbers of *B. dorsalis* and *B. correcta* counted. The data was converted to the number of Flies per Trap per Day (FTD) for the respective months over which population monitoring was conducted.

Fruit fly damage to dragon fruit was assessed by randomly sampling at the harvest stage, dragon fruits from the core zone (160 fruits), buffer zone (480 fruits) and farmer's practice zone (480 fruits). Fruits were then held individually in plastic containers over sterilised sawdust and checked for collection of pupae and emergence of adult flies.

Fruit fly damage to seven other alternate host fruits found commonly growing in the area in the three zones (rambutan, rose apple, custard apple, guava, Barbados cherry, jujube and mango) was assessed by collecting at random 100 ripe fruits of each species. The fruits were then transported to the laboratory where they were held individually over sterilized sawdust in plastic containers and monitored for the collection of pupae and emergence and adult flies. The fruits were scored as damaged when one or more pupae were detected, or scored as undamaged if no pupae were produced.

## Results and Discussion

### *Fruit fly host fruit survey in Binh Thuan*

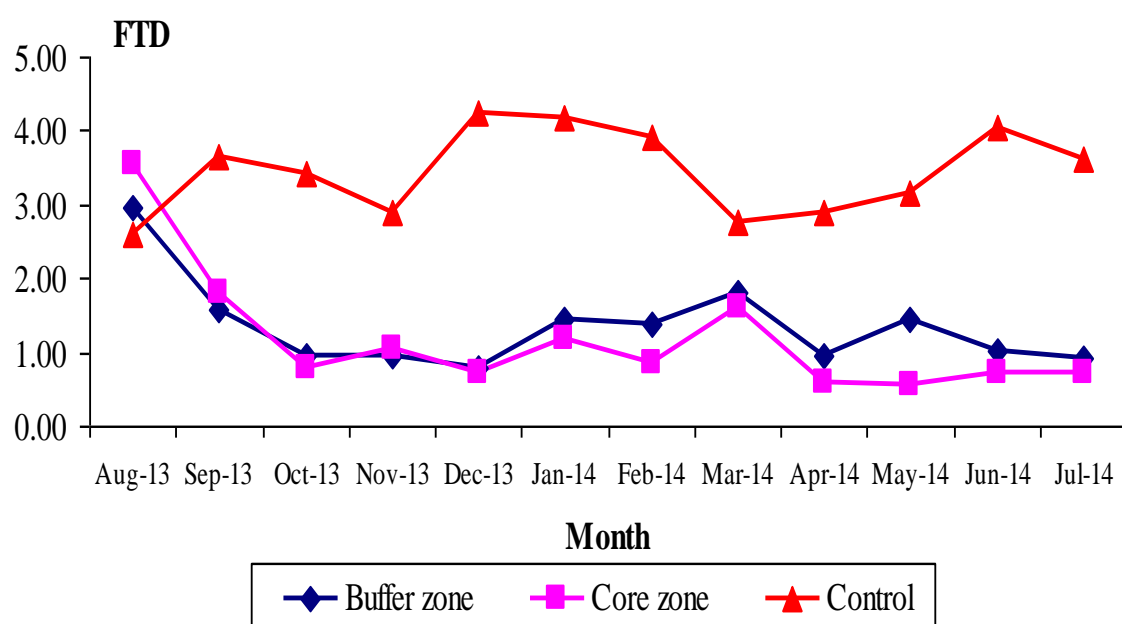
Results from collecting damaged fruit from January to December 2013 found that there are 15 fruit species attacked by *B. dorsalis* and *B. correcta* (Table 1). Both red flesh and white flesh dragon fruit species were attacked by both species of *Bactrocera*. This result is consistent with research of Nguyen et al. (2011).

**Table 1.** Fruit found to be infested with *Bactrocera dorsalis* and *Bactrocera correcta* in Binh Thuan providence, Viet Nam from January to December 2013.

Common name	Scientific name	Month	<i>B. dorsalis</i>	<i>B. correcta</i>
Rambutan	<i>Nephelium lappaceum</i>	5, 6, 7	+	
Cashew	<i>Anacardium occidentale</i>	4, 5, 6	+	+
Papaya	<i>Carica papaya</i>	9, 10, 11	+	
Rose apple	<i>Syzygium malaccensis</i>	4, 5, 6, 7	+	+
Star fruit	<i>Averrhoa carambola</i>	5, 6, 7, 8		+
Custard apple	<i>Annona squamosa</i>	6, 7, 8	+	+
Guava	<i>Psidium guajava</i>	4, 5, 6, 7	+	+
Barbados cherry	<i>Malpighia emarginata</i>	3, 4, 5, 6, 7	+	+
Jujube	<i>Ziziphus mauritiana</i>	6, 7, 8, 9	+	+
Mango	<i>Mangifera indica</i>	4, 5, 6, 7, 8	+	+
Milk fruit	<i>Chrysophyllum cainito</i>	1, 12	+	
Red flesh Dragon fruit	<i>Hylocereus polyrhizus</i>	6, 7, 8	+	+
White flesh Dragon fruit	<i>Hylocereus undatus</i>	1, 2, 6, 7, 8, 11, 12	+	+
Jamaica cherry	<i>Muntingia calabura</i>	5, 6, 7	+	
Terminalia	<i>Terminalia catappa</i>	6, 7, 8	+	
<b>Total of fruit species infested</b>			<b>14</b>	<b>10</b>

### *Effectiveness of area-wide suppression fruit fly in Binh Thuan*

Fruit fly populations as indicated by the number of *B. dorsalis* and *B. correcta* in methyl eugenol traps were lower in the core zone and buffer zone compared to farmer's practice zone (Fig. 1). In the core zone FTD decreased from 3.5 on August 2013 a low of 0.7 on July 2014. In the buffer zone, the FTD decreased from 3.0 on August 2013 to 0.9 on July 2014. In the control zone (farmer's practice), the FTD was 2.6 at the start of the experiment in August 2013 and remained consistently above this value for the rest of the experiment until July 2014.



**Fig. 1.** Number of *Bactrocera correcta* and *Bactrocera dorsalis* adults per trap per day in methyl eugenol traps in Binh Thuan province, Viet Nam (August 2013 to July 2014).

During most of the second crop season (with electric light to stimulate flowering; fruit sampled from October to January), the average percentage of dragon fruit damaged was 2.9% in the core zone, 3.5 % in the buffer zone and 5.9% in the farmer practice zone (Table 2).

**Table 2.** Percentage damaged dragon fruits during the second cropping season of dragon fruit sampled from the core, buffer and farmer's practice (control) zones in Binh Thuan province, Viet Nam.

Month	Core zone		Buffer zone		Control	
	Total fruit	Damaged percent (%)	Total fruit	Damaged percent (%)	Total fruit	Damaged percent (%)
Oct-13	160	4.4	480	5.4	480	7.5
Nov-13	160	3.1	480	5.0	480	7.3
Dec-13	160	1.9	480	4.0	480	5.6
Jan-14	160	2.5	480	1.9	480	4.4
Feb-14	160	2.5	480	1.5	480	4.6
<b>Average</b>	<b>160</b>	<b>2.9</b>	<b>480</b>	<b>3.5</b>	<b>480</b>	<b>5.9</b>

In part of the main fruiting season from June to September, fruit samples were analyzed and the percentage of damaged dragon fruit in the core zone and the buffer zone was found to be

lower than in the farmer's practice zone for each month sampled and the average damage was 2.5, 5.7 and 9.9 % in the core, buffer and farmer practice zones respectively (Table 3).

**Table 3.** Percentage of damaged dragon fruits in the main crop season of dragon fruit from the core, buffer and farmer's practice (control) zones in Binh Thuan province, Viet Nam.

Month	Core zone		Buffer zone		Control	
	Total fruit	Damaged percent (%)	Total fruit	Damaged percent (%)	Total fruit	Damaged percent (%)
Jun-14	160	3.1	480	7.5	480	10.6
Jul-14	160	3.8	480	7.1	480	12.9
Aug-14	160	1.9	480	5.6	480	10.2
Sept-14	160	1.3	480	2.5	480	5.8
<b>Average</b>	<b>160</b>	<b>2.5</b>	<b>480</b>	<b>5.7</b>	<b>480</b>	<b>9.9</b>

Besides collecting dragon fruit, we also collected other fruit in the demonstration area (including both core and buffer zones) and farmer's practice zone to evaluate damage. Results show that other fruits in the demonstration area have consistently lower damage rates than in the farmer's practice zone, especially in Barbados cherry, mango and guava. In the demonstration area, the heaviest damage was in guava (14%), mango (18%) and in Barbados cherry (31%). In the farmer's practice zone, the damage for these fruits was 56%, 65% and 71 % respectively (Table 4).

**Table 4.** Percentage fruit fly infested fruit out of 100 ripe fruits sampled from alternate host fruit trees from the demonstration sites (core and buffer zones combined) and farmer practice zones in Binh Thuan province, Viet Nam in 2014.

Common name	Total fruit	Infestation (%)	
		Demonstration sites	Farmer's Practice
Rambutan	100	6	20
Rose apple	100	15	42
Custard apple	100	0	8
Guava	100	14	56
Barbados cherry	100	31	71
Jujube	100	19	29
Mango	100	18	65

In the first year of the area-wide fruit fly suppression demonstration project in Binh Thuan, which used MAT, weekly bait sprays during the dry season or protein baits in bottles traps during the rainy season and sanitation in the core zone, and MAT and sanitation in the buffer zone, resulted in lower populations of fruit flies than in areas that used standard farmer's practices. The number of flies captured and percentage of infested fruit in the demonstration zone (core and buffer zones combined) was always lower than in the farmer's practice zone. These results can be used to provide a scientific base to expand the area-wide suppression for fruit fly control in Binh Thuan, Viet Nam. Such an area-wide fruit fly suppression project would also help farmers reduce the use of insecticide cover sprays. The incorporation of the sterile insect technique integrated with the studied suppression methods as part of an AW-IPM strategy is being considered in the future and in the future would allow for the creation of areas of low pest prevalence, which in turn would reduce insecticide application and fruit residues, improve dragon fruit value and expand export markets for dragon fruit growers in Viet Nam.

### Acknowledgements

We thank Ham Hiep (farmer), Binh Thuan Department of Agriculture and Rural Development for cooperating and supporting us in implementing Area-Wide Suppression of *Bactrocera* Fruit Flies in Dragon Fruit Orchard in Binh Thuan, and FAO/IAEA through the project VIE 5017 for technical assistance and supporting the implementation of the project.

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## Communication codes to win the medfly battle

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### Abstract

*Background:* Achieving fruit fly suppression under an Area Wide Management (AWM) approach requires the implementation of coordinated and consistent control actions with a high level of participation of fruit growers and the general public. A high level of understanding of the insect pest's lifecycle to reduce its population is key under an integrated management approach: it determines the treatments that will target the pest more effectively throughout its lifetime. Under the lifecycle focus, the mosaic of fruit types and maturity adds a complexity to AWM. Addressing the inconsistent actions of both growers and public to control medfly requires a review of how much of the lifecycle concept is understood by both groups, and an understanding of the factors influencing people's adoption of medfly control.

*Material and methods:* a) Evaluation of communication formats and context in which the lifecycle information is used in successful AWM programs, b) Survey commercial growers' and backyard owners' perceptions of the lifecycle and the factors that may be affecting their adoption rates to control medfly in the southwest of Western Australia (under the Theory of Planned Behaviour framework).

*Results:* From a sample of 605 people from Western Australia, approximately 66% of respondents were able to indicate that targeting the adult stage of medfly was the most important stage for control. However, only 39% were able to indicate the importance of controlling fruit fly all year round. This may be explained by people associating the time of control with the fruit species they grow on their property or their familiarity to fruit species susceptible of medfly attack. Such result indicates that the lack of association of control with other fruit trees probably present in the same area is an issue to accomplish AWM. The review of some communication material showed that the fruit fly lifecycle is used in different contexts, but the relationship between lifecycle, control method and time of application is hardly made evident. In many cases the lifecycle is not included in the information produced.

*Discussion:* Research into people's understanding of the medfly lifecycle and its association with current control methods will help in the designing of an effective communication strategy for AWM and improve the participation rates of growers and public in the fight against medfly. Successful communication only occurs when the sender and the recipient share a common set of codes and signs, and a common set of rules that help in the understanding of the meaning of those codes within a context. It is anticipated that knowledge

gained from understanding the medfly lifecycle and its implications for fruit industry will provide growers and public with the necessary codes to understand when and how to target fruit fly, but most importantly why it is needed to be done in such way.

*Keywords:* Area Wide Management, lifecycle, Mediterranean fruit fly, medfly, science communication.

## Introduction

In 2001, twenty-nine Navajo Code Talkers were honoured by the United States Congress for language skills that helped the United States win World War II (CNN, 2001). Using lesser-spoken languages, Code Talkers were able to communicate and transmit messages through codes built upon their native languages. Their codes, only known to them, were never decoded by Japanese military, therefore conducive to the victory of the United States in many battles in the Pacific (Civic Impulse, 2016).

Language, code and code-breaking are part of people's everyday communication process. Communication is the act of conveying intended meanings from one entity or group to another through the use of mutually understood signs (such as language) and which are interpreted by shared codes, or a set of conventions, to communicate meaning (Danesi, 2004; Leeds-Hurwitz, 2012). Since the meaning of a sign depends on the code within which it is situated, codes provide a framework within which signs make sense. Consequently, interpreting signs requires familiarity with the sets of conventions or codes to communicate meaning (McFarlane, 2010) and to create shared understanding.

To meet with the requirements of an economy driven agricultural industry, we need to improve how people become knowledgeable in agricultural issues and the way we communicate such knowledge (Lundy, 2005). Science-based government institutions are advised to communicate not only broad policy directions, but also data-rich, leading-edge science concepts, layperson interpretations, and clear advice for action and behaviour change. However the range of ways of talking about the purposes and content of science communicated to the public are predominantly framed as a one-way communication process (McFarlane, 2010). As a result, communication is difficult because it is hard to interest the public in the research if it is not directly relevant or applicable to them (Davies, 2008). Is in this sense that science communication helps provide educational and interpretative opportunities for the general public to better familiarize itself with science. Codes are built through the use of analogies and metaphors which help discuss science topics by setting up a simple correspondence between them (Halkia & Mantzouridis, 2005).

Burns et al. (2003) indicated that to increase the public's engagement, science communication has to focus on enhancing public scientific awareness, literacy, and culture by building enjoyment, interest, opinion-forming, and understanding of science in its participants. Science communication also provides skills, activities, and dialogue to enable the general public, policy makers, and science practitioners to interact with each other more effectively. Public understanding of science is a combination of the comprehension of scientific facts, ideas and

policies, with a knowledge of the impact such facts, ideas and policies have “on the personal, social and economic well-being of the community” (Bryant, 1998; Bryant & Stocklmayer, 2000). Despite the differing perceptions, knowledge, and responsibilities of scientists and the public, it is the science communicator that has been expected to provide the link between science and the public. The benefits from the role of the science communicator derived from helping people understand science is observed in increased public support (Lundy, 2005) and participation.

### *Controlling fruit fly without chemicals in Western Australia*

Traditionally, fruit flies, like many insect pests of economic importance, were controlled with pesticides. Changes in the attitudes towards the use of pesticides in agricultural and horticultural practices has evolved as a result of the increased awareness on how chemicals used in control, management or eradication of insect pests may adversely impact food quality, the environment, and human health.

In Australia, fruit flies were controlled historically on properties with the pesticides dimethoate and fenthion, organophosphate cover sprays that kill insect pests systemically or by contact (Dominiak & Ekman, 2013). Following extensive reviews on the risks to human health and the environment posed by the chemical residues left behind from dimethoate and fenthion in fruit and vegetables, the Australian Pesticides and Veterinary Medicines Authority (APVMA) suspended the use of dimethoate in 2011 and imposed severe restrictions to the use of fenthion in 2013 (APVMA, 2011, 2013; DAFWA, 2011). Since 2012 fenthion was no longer available for household usage and a complete ban in commercial orchards was enforced in 2015. During the suspension period, the Hills Medfly Initiative was created as a working group with the participation of growers, Department of Agriculture and Food, Western Australia, (DAFWA), local government representatives and industry stakeholders in the Perth Hills in 2014. The initiative identified issues and priorities that needed to be addressed to help the industry transition away from using the chemical fenthion. The strategy was outlined with an Area Wide Management (AWM) perspective to promote the adoption of fruit fly control by working closely with growers to achieve control on their properties, and included a communication strategy to enable the change management process for medfly management (DAFWA, 2014a, 2014b).

The increasing medfly population in the southwest of WA reflect the lack of collective participation of all fruit growers. Protecting WA’s fruit industry from the Mediterranean fruit fly, medfly, (*Ceratitis capitata*) prompted DAFWA to implement the use of Area Wide Management (AWM) for this species in urban and peri-urban areas where proximity to fruit production areas occurred. AWM is an integrated pest management approach supported by a coordinated, sustainable and preventative strategy that targets the entire pest population within a defined geographical area (Faust, 2008). While it is DAFWA’s role to regulate the management of fruit fly and to encourage fruit grower’s participation in AWM, it is the responsibility of property owners to take the prescribed measures to control medfly on their own properties (BAMA ACT, 2007). AWM strategy is designed to include practices that



control pests while reducing the use of pesticides (Hendrichs, et al. 2007; Utah State University, 2001), analogous to an integrated pest management strategy but which focuses on a broader spatial scale (FFTC, 2006; Hendrichs et al., 2007). Practices include property hygiene, baiting and trapping activities as key control action on properties.

Understanding how the pest interacts with their hosts, the landscape and climatic conditions is important to determine the activities to follow for effective control and/or eradication of the pest (FAO 2014a, 2014b). This knowledge helps managers and affected users to apply the best control methods most suitable to each life stage of the pest, therefore, improving the efficiency and effectiveness of the management strategy (Dauer et al., 2012; Elliot et al., 2008). Hence, medfly populations are best controlled by targeting the adult stage to stop them from breeding which will prevent the development of the eggs into the larval stages that damage the fruit. Additionally, the control of the fruit fly larvae in infested fruit requires strict procedures to minimize the risks of re-infestations. This is achieved by preventing the larvae reaching maturity and emerging from the fruit to develop into the pupae stage (DAFF, 2007; HAW-FLYPM, 2014). However, without cover sprays or other safe chemical option to destroy the larvae in fruit and the pupae in the ground, then targeting the adult stage for control is extremely important.

#### *Communicating science for fruit fly control*

Worldwide, fruit fly integrated pest management and AWM programs (Asian Fruit Fly IPM Project, 2011; Barnes et al., 2006; DAFF, 2007; Dominiak & Commbes, 2010; SENASA, 2007) have incorporated the biology and ecology of fruit fly species in communication materials. Training workshops and field demonstration plots are effective science communication tools (Silva & Bultitude, 2009) that can help to improve people's understanding of the link between fruit fly lifecycle, host plant development and control techniques. These activities are part of an ongoing awareness campaign aimed to increase the participation of the public and the effective management of fruit fly on their properties under AWM (CPS, 2001; DAFF, 2007; Mau et al., 2007; MOSCAMED, 2014).

In WA, DAFWA produced a series of information sheets, web pages and media releases to increase public awareness on fruit fly issues. However a review on how the state deal with the impacts of biosecurity pests in general (Government of Western Australia, 2013) found that state agencies dealing with biosecurity pests do not make use of all the potential information sources to enhance public awareness. As a result the information is not easy to find, is not delivered in a timely manner, it is hard to follow and in some cases it lacks any advice or options to control the pest. The report found, in general, information on pest threat, pest impact and spread of pests was insufficient and sometimes unclear.

Breaking the medfly lifecycle in an AWM situation without organophosphate chemicals, requires commercial and backyard fruit growers to apply a coordinated set of control treatments. This means fruit growers will require a better understanding of the relationship between the lifecycle, fruit growth development and control methods to gain full control in

the future.

The objective of this research was to assess current knowledge of commercial growers, ‘hobby farmers’ and backyard owners from urban, peri-urban and rural areas in WA about basic fruit fly biology, fruit fly control methods and the role knowledge plays in an individual’s intention to control fruit fly.

## Material and Methods

A state-wide survey constructed under the TPB was prepared to collect, among others, information regarding people’s understanding basic medfly scientific facts as well as people’s attitudes and perceptions around medfly control methods (Annex). The present study draws data from a survey evaluating Western Australian’s intention to control fruit fly. The data collected was used to determine the role of knowledge in the intention to control fruit fly, and to evaluate the knowledge of key scientific facts to control fruit fly. The objective was to measure the influence of subjective norms, attitudes towards and perceived behavioural controls regarding on how users the perception of efficiency, effectiveness and complexity of the control treatments affected their intention to control fruit fly.

The section regarding scientific information was constructed to assess:

- a) knowledge of the medfly lifecycle and its relation with control methods,
- b) perceived efficacy of control methods of the different medfly life stages, and
- c) perceived factors affecting the adoption of the different medfly control methods.

### *Survey distribution*

Participants in the first survey (N=598) were drawn from across WA. The survey was administered between November 2013 and March 2014. The survey was made available via the Internet and notifications about the survey, with an invitation to participate were sent through emails including community gardens groups, ethnic and cultural associations, fruit industry groups and gardening social media and news media networks. Additionally, hard copies of the survey were delivered to and collected from home properties from randomly selected street blocks in the suburbs of Willetton, Jarrahdale, Highgate and Bridgetown.

### *Survey questions*

Respondents were asked multiple-choice questions regarding

- a) the best time to control fruit fly on properties (Summer, Autumn, Winter, Spring, All year round, Don’t know); and b) the most important developmental stage to control fruit fly (Egg, Larvae, Pupae, Adult, All stages, Don’t know).

Respondents were also asked to provide opinions on decisions and confidence influenced by the perceived characteristics of the control methods. The response choices for perception questions were given on a five-point Likert-type scale according to levels of influence of the

characteristics of control into an individual's decision to control (1= Not at all; 5=Extremely) and according to levels of confidence to control based on the characteristics of treatments (1= Not at all confident; 5=Extremely confident).

### *Data Analysis*

Statistical analysis of the questions regarding knowledge within the state-wide survey were calculated based on valid cases using STATA (13.1) software. The results presented here correspond to the percentage of respondents that chose a particular answer therefore the sums for each group do not add 100%.

For analysis purposes, answers regarding timing to control were first coded as follows: 'All year round' and 'Adult stage' were coded 1 for "correct"; all other responses were coded 2 for "wrong". Responses were then added next to create a knowledge index, which indicated the respondent having Correct knowledge=2; Partial knowledge=3; and Incorrect knowledge=4.

A linear regression was used to predict individuals' intention to control fruit fly on their property from their attitudes, subjective norms, perceived behavioural control, and knowledge of key aspects governing fruit fly control.

$$\text{Intention} = \sum \text{Attitudes} + \sum \text{Subjective Norms} + \sum \text{Perceived Belief Control}$$

Based on TPB (Ajzen, 2006) we evaluated intention using direct constructs to attitudes, and to control belief and control belief power (as components of perceived behavioural control) and the calculated knowledge index. Results also present information on proportions and frequencies of responses.

Data were analysed to measure the association of independent variables identified as attitudes (the control was perceived as worth, complex, efficient, effective, demanding, or expensive), subjective norms (the influence of neighbours, fruit growers or others) and perceived behavioural controls (confidence to be able to control if control is seen as slow, irregular, complex, toxic or expensive— in the intention to control medfly – as dependent variable. The analysis was applied to group factors, such as property type, occurrence of fruit growing activity, occurrence of fruit fly control by groups within the sample growing fruit trees and involved in active control.

## **Results and Discussion**

We collected a total of 598 responses from all of the state, with a large concentration in the south west of WA where the medfly is endemic (Fig.1).

Growing fruit trees in backyards in urban, peri-urban and rural areas in WA is very common (Wise, 2014). From the large proportion of respondents (83%) that grow fruit trees on their properties data indicated 81% grow fruit trees in backyards and front yards while 18% grow fruit trees in orchards and/or hobby farms. The proportion of individuals controlling fruit fly on their property (57%) is slightly higher than those not controlling for it (43%) (Table1).



**Fig. 1.** Map of the 141 post code locations from respondents to the medfly survey.

**Table 1.** Distribution of respondents growing fruit trees and implementing medfly control in the whole sample and against the location of fruit trees on their property.

	Overall sample (%)	By type of property (%)		
		House	Orchard	Other*
Grow fruit on property	83.4	81	18	1
control medfly	5	44	12	1
do not control medfly	43	37	5.5	0.5

\* community garden, apartment balcony.

*Q. What developmental stage is most important to control fruit fly?*

A large proportion of individuals believed that the adult and egg stages were the life cycle stages that can control medfly more effectively (Table 2). Overall, 64% of all respondents identified the adult stage as the most important stage to control. The adult stage was identified by a large number of respondents that grow fruit and by those that grow fruit and control fruit fly. However 63% within the respondents not growing fruit and 55% of those growing fruit but not controlling indicated the egg as the most important stage to control fruit fly.

**Table 2.** Proportion of respondents (%) and the stage believed as being the most effective to control medfly.

Whole sample	Egg	Larva	Pupa	Adult	Don't know
All respondents (N=598)	60	52	43	64	17
Not growing fruit (N=95)	63	46	32	42	22
Growing fruit (N=479)	62	56	47	72	17
controlling fruit fly (N=271)	68	67	58	87	9
not controlling fruit fly (N=208)	55	42	33	51	26

*Q. How effective are each of the following methods to reduce fruit fly numbers?*

Responses to the question indicated that despite the ban of fenthion in households, and the restrictions for its use in commercial enterprises, spraying was perceived as an effective to very effective method to control medfly (Table 3). This preference highlights the potential problem to achieve the chemical free situation sought with an AWM approach as traditional cover sprays continue to be seen as an effective and quick treatment against medfly.

**Table 3.** Respondents controlling fruit fly (%) and their perception of the effectiveness of control treatments (N=274).

Treatment	very ineffective	ineffective	neither	effective	very effective
Removing fallen	3	5	6	48	31
Composting	35	29	3	12	2
Burying	30	23	7	19	3
Baiting	3	5	9	54	23
Spraying	2	2	5	34	38

*Q. Fruit fly stage(s) targeted by each control method*

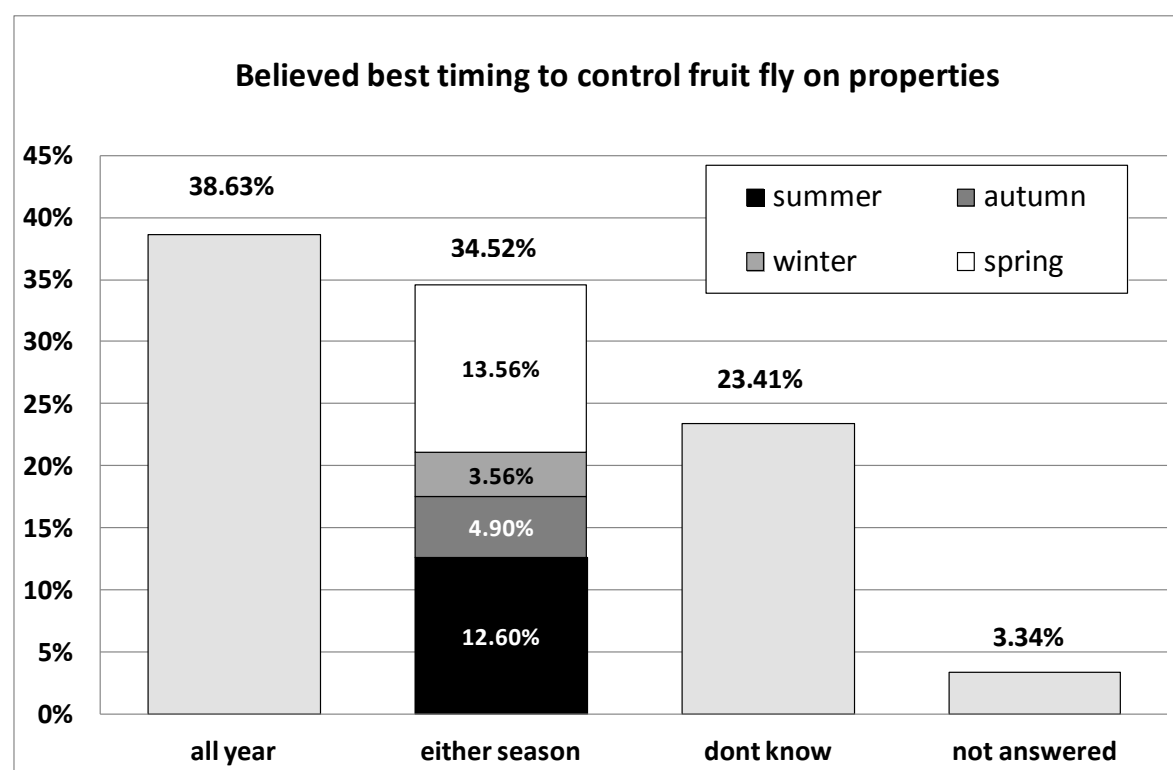
Responses indicate that fruit growers made the right associations between the treatments promoted as necessary to control fruit fly and the life stages the treatment is supposed to control. Property hygiene in fruit growing areas is always an issue of concern for commercial fruit growers because lack of hygiene has the potential to sustain or spread pests and diseases. The selection to remove and bury infested fruit to target larval stages (Table 4) can break the medfly life cycle by killing any larvae present and preventing re-infestations. However, responses indicating composting as being an effective method to control fruit fly (Table 3) reveal the need to develop strategies to train users on how to dispose infested fruit. A reason of this choice may be that people believe ‘composting untreated infested fruit’ is a safe choice that controls effectively medfly populations while it is safe to the environment.

**Table 4.** Medfly life stages targeted by individual control treatments. Proportion of responses (%) expressed by fruit growers actually controlling medfly (N=213).

Control treatment	Egg stage %	Larva stage %	Pupa stage %	Adult stage %	don't know %	Total
Removing fallen	27	33	23	14	3	100
Burying	23	31	18	9	19	100
Spraying	22	24	14	31	9	100
Trapping	3	3	3	83	8	100
Baiting	5	4	2	80	9	100

*Q. What is the best time to control fruit fly?*

Approximately 39% of all respondents indicated it was important to control medfly year round while other respondents suggested summer and spring were the right times to control medfly (Fig.2). This type of response may be a result of people's previous experience growing seasonal fruit. Only 54% of all respondents who apply control indicated that all year was best to control fruit fly (Table 5), while the proportion for those growing fruit in households and orchards, the Fig.was 53% and 38%, respectively. In general winter was the least preferred season to implement control.



**Fig. 2.** Distribution of preferences for the effective time to control medfly from the whole sample (N = 598).

**Table 5.** Seasons important to control (%) by groups.

Group	Summer %	Autumn %	Winter %	Spring %	All year %	Don't know %
All respondents	24	9	4	30	40	24
Not growing fruit	8	3	8	26	36	30
Growing fruit	27	11	3	31	41	23
not controlling fruit fly	17	6	2	29	24	45
controlling fruit fly	33	14	4	33	54	7
house control	25	8	3	31	38	26
orchard control	34	23	2	34	53	11

*Note:* Results to multi-choice questions in the form of percentage of respondents that selected the answer.

### *Knowledge and intentions to control fruit fly*

Data from the stages to effectively control fruit fly and timing were combined to build a fruit fly knowledge index and compared between all respondents, and the groups of growers who either controlled fruit fly on their property or not. The results showed that a very low percentage of individuals obtained a correct knowledge (10%, 15% and 6 % respectively) indicating that few people really know what to do and when to control fruit fly in an effective manner (Table 6).

**Table 6.** Knowledge degree regarding season and life stage (% of respondents).

Group	Correct knowledge %	Partial knowledge %	No knowledge %
All respondents	10	43	47
Not controlling fruit fly	6	33	61
Controlling fruit fly	15	56	29

*Note:* analysis based on valid cases.

These results suggest that fruit growers need to improve their understanding of how fruit trees outside their property impact on the fruit fly problem on their own property. Information and training has to be developed to make evident the connection between medfly development and fruit growth development in a larger context outside the individual property.

A result of concern is fruit grower's perception that spraying is an effective method able to target all life stages. Between 20 to 26% of people who grow fruit believe that spraying can control all life stages and 65% of these consider that spraying is an effective treatment to control medfly (Table 3 and Table 4).

Spraying with cover sprays has been considered a rapid method to deal with fruit fly infestations (Sproul et al., 2002). Spraying is also less labour intensive compared to the requirements for baiting and trapping. The advantages provided by spraying methods help to explain why people are frustrated with the removal of traditional cover sprays.

A comparison of how the knowledge of correct stage and season to control may affect the intention to act is presented in Table 7. The perceived controls of complexity and effectiveness and the knowledge are highly significant for the whole sample (N=522) and for fruit tree owners (N=442). The overall results indicate that knowledge ( $P < 0.001$ ) is important as a predictor of whether people do or do not apply control – indeed, it is by far the biggest predictor. However, knowledge is not significant for those who apply or do not apply control.

Overall, people's limited knowledge of fruit fly control methods and its relationship on fruit fly lifecycle may have implications on how people perceive themselves participating in the control of medfly. From these regressions and from the results in Table 5, respondents within the group that applies control for medfly probably have greater knowledge of fruit fly biology or understand how to apply the control treatments therefore knowledge may no longer be a predictor of intention. On the other hand, the group of respondents that don't control for medfly all have equally low knowledge therefore knowledge is not a predictor of intention. Those who control fruit fly may be influenced by the complexity and the effectiveness of the control treatment while those that do not control may be influenced by the speed of the treatment as well as their confidence on the complexity of the treatment. In all groups the attitude towards effectiveness of the control is significant to highly significant.

Improving the knowledge and understanding of the fruit fly biology will help decode the requirements of control treatments at specific time and improve the participation of individuals in control behaviour. Elsey and Sirichoti (2001) indicated that adoption and uptake is influenced by the actual characteristic of the innovation, which corresponds to the knowledge and understanding of the technology's useful qualities. Key aspects of pest management for medfly control relate to both biological and ecological interactions: a) the life cycle of a fruit fly varies considerably between the seasons and it can be affected by changes in temperature and humidity; b) medfly has the capability to overwinter as adults, as eggs and larvae inside fruit or as pupae buried in the ground (Broughton, 2012) - the latter is the most difficult stage to control as the pupae can be buried deep within the ground and can only be killed with harsh chemicals that impact on soil ecology; c) distribution of fruit fly populations is related to the availability of host tree species which provide food and shelter; d) moisture is the major limiting factor of fruit fly populations during summer (Dominiak et al., 2006); however, artificial conditions created by irrigation within backyards, orchards and landscaped areas have increased the ability of fruit fly to survive well beyond the summer season.



**Table 7.** Multivariate linear regression analysis of individuals' intention to control medfly.

<b>Behavioural Constructs</b>	<b>Statewide (N=522)</b>		<b>Have (N=442)</b>		<b>trees Apply (N=255)</b>		<b>control Don't (N=187)</b>		<b>control</b>	
	<b>Coef.</b>	<b>P&gt;t</b>	<b>Coef.</b>	<b>P&gt;t</b>	<b>Coef.</b>	<b>P&gt;t</b>	<b>Coef.</b>	<b>P&gt;t</b>	<b>Coef.</b>	<b>P&gt;t</b>
<i>Attitudes</i>										
Demand	-0.03	0.497	-0.03	0.511	-0.03	0.380	0.08	0.233		
Complexity	-0.01	0.783	-0.04	0.393	-0.02	0.601	-0.05	0.506		
Effectiveness	-0.07	0.020*	-0.09	0.004*	-0.10	0.000**	-0.11	0.031*		
Subjective Norm	0.10	0.005*	0.07	0.054	-0.02	0.658	0.00	0.979		
<i>Behavioural controls</i>										
Influence speed	0.07	0.141	0.07	0.195	0.04	0.314	0.19	0.031*		
Influence complexity	-0.24	0.000**	-0.23	0.000**	-0.09	0.038*	-0.18	0.064		
Influence effectiveness	0.24	0.000**	0.22	0.001*	0.20	0.000*	-0.06	0.566		
Confidence if slow	0.12	0.098	0.12	0.112	0.02	0.701	0.24	0.061		
Confidence if complex	-0.09	0.249	-0.07	0.390	0.07	0.242	-0.31	0.017*		
Confidence if irregular	0.04	0.596	0.04	0.625	0.03	0.603	0.19	0.112		
Knowledge index	-0.38	0.000**	-0.38	0.000**	-0.05	0.469	-0.12	0.388		

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; *Note:* analysis based on valid cases.

Science communication can influence how the public perceives risks (Price, 2001; Savadori et al., 2004) as knowledge can lead to changes in the public's views, attitudes and behaviours thus impacting society. The acquisition and delivery of knowledge can be better focused to 'recruit' the public to support agriculture biosecurity messaging as the public can assist with pest problems in ways which are temporal and spatially achievable, ecologically and economically sound and which satisfy the perception of well-being (Di Guiseppe et al., 2007; Dike et al., 2006; Price, 2001). From the policy perspective, increased knowledge could mean better pest management practices and outcomes (Price, 2001). For relatively simple innovations, a landholder's probability of making a good decision increases over time with increasing practical knowledge (Pannell et al., 2006).

## **Conclusions**

For an AWM fruit fly strategy be successful it requires all fruit growers to participate in fruit fly control activities. To truly achieve this goal individuals require capability in understanding how to manage the problem as well as an understanding of the consequences of their involvement, or the lack thereof when undertaking control practices. However, an individual's intention to participate may be affected by his/her perceptions of inconvenience or disappointment – probably a reflection of the individual's reduced or inexistent satisfaction with the outcomes of the control treatment.

The results presented here indicate that when people do not have a clear understanding of the biology of the fruit fly and how to interpret this information in relation to the fruit development on their own properties, then it is more likely that control treatments will fail. Scientific evidence provides information to interpret the natural processes such as dispersal and reproduction surrounding medfly adaptation and survival. Facilitating the communication of such processes will improve awareness of the medfly problem and its solution. The results from this study can help communication strategies address issues regarding the medfly lifecycle identified as necessary to improve the adoption of medfly control methods. Determining the fruit grower's knowledge gaps regarding the fruit fly lifecycle in the management process will help improve the quality of information necessary to increase fruit growers' capability to manage medfly on their properties.

The results of this research provide a baseline to better understand the role increased knowledge can play in the adoption of fruit fly control practices. It also reflects the need to review fruit fly communication strategies in WA and of the way in which science is communicated to engage people to help solve the medfly problem. It highlights the need of rethinking the quality of information we disseminate to help increase scientific knowledge which in turn will provide the necessary communication codes to help interpret and understand the requirements to achieve fruit fly management but most importantly will provide a rationale for management actions.

## Acknowledgements

The authors would like to acknowledge the support of the Australian Government's Cooperative Research Centres Programme.

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## Annex. Medfly Motivations survey questionnaire



THE UNIVERSITY OF  
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RESOURCE ECONOMICS

The purpose of this questionnaire is to collect information to explore perceptions and beliefs about fruit fly control, whether you have fruit trees or not on your property. Understanding how you and other people think about fruit fly control will help create strategies to increase community participation in rural, outer metropolitan and metropolitan areas in Western Australia.

Increasing adoption of control methods will help fruit growers and backyard owners to reduce the fruit fly population and help Western Australia maintain its sustainable and internationally competitive fruit industry.

Many questions in this survey make use of rating scales; you are asked to select the choice that best describes your opinion. Some of the questions may appear repetitive, but they address somewhat different issues that may be involved in the adoption of control methods.

Participation in this study is voluntary and anonymous and all data collected will be treated as confidential. This survey is part of a PhD study at the University of Western Australia regarding the management of Mediterranean fruit fly in Western Australia. This survey has been approved by the UWA Human Ethics Committee (RA/4/1/6047).

To thank you for your participation, you have the option to go into a draw to win one of two \$100 vouchers from Coles or Woolworths. Entry details are provided below.

The survey will take 20 to 30 minutes. Please read each question carefully and answer it to the best of your ability. There are no right or wrong answers. We just want to know your personal opinions.

Thanks again for your participation.

✂-----✂-----✂-----✂-----✂-----✂-----✂-----

To enter the prize draw for one of two \$100 dollar vouchers from COLES or WOOLWORTHS, please detach this section and send it together with your completed survey to:

**Medfly survey**

Science Communication, School of Animal Biology, M092  
The University of Western Australia  
35 Stirling Hwy  
Crawley 6009

Tell us your name and the best way to contact you:

☐ Telephone / mobile:

☐ Email:

☐ I would like to receive information on Mediterranean fruit fly

*(These details will not be used for any purpose other than to notify the winner or to send information on medfly.)*

**Demographic information**

**Gender**      ☐ Male      ☐ Female

**Age range**      ☐ less than 25 years      ☐ 25 - 34 years      ☐ 35 - 44 years  
                          ☐ 45 - 54 years      ☐ 55 - 65 years      ☐ more than 65 years

**Postcode**

**This questionnaire has been prepared to collect information on people's views regarding the control of fruit fly on properties in Western Australia.**

Properties include households, backyards, frontyards, verges, farms and orchards.

**1. Do you have fruit trees on your property?**

- ☐ Yes  
☐ No (If no, skip to question 6)

**2. Please indicate where the majority of your fruit trees are located**

- ☐ house property (owned or rented; suburban, semi-rural or rural)  
☐ orchard / farm  
☐ other

**3. What type of fruit do you have on your property? (select all that are appropriate)**

Fruit variety	Number trees/plants	Area planted
<input type="radio"/> citrus (i.e. orange, mandarine, lemon, lime, cumquat, tangelos, grapefruit)		
<input type="radio"/> stonefruit (i.e. peach, nectarine, apricot, cherry)		
<input type="radio"/> avocado		
<input type="radio"/> grape		
<input type="radio"/> other: <input type="text"/>		

(please indicate what other fruits)

**4. Do you apply any measures on your property to control fruit fly?**

- ☐ Yes  
☐ No (If no, skip to question 6)

**5. What motivates you to control fruit fly on your property? (you may select more than one answer)**

- ☐ Increase markets for fruit tree growers  
☐ Reduce my expenses to produce fruit  
☐ Help grow more fruit trees without worries  
☐ Provide clean and healthy fruit  
☐ Provide a nice and neat area to enjoy  
☐ Help eliminate fruit flies in my area/region  
☐ Other:

(please indicate what other reasons)



6.

**Mediterranean fruit fly (medfly) occurs in Western Australia.**





Fruit fly survival depends on the presence of fruit to breed and multiply.

Fruit fly larvae damage fruit as they develop inside it causing fruit to ripen prematurely and rot.

**When do you think is the best time to control fruit fly on properties?***(you may select more than one answer)*
☐ summer    ☐ autumn    ☐ winter    ☐ spring    ☐ all year round    ☐ don't know

7. In your opinion, what developmental stage is most important to control fruit fly?

*(you may select more than one answer)*

					
1mm long	8 mm long	4 mm long	3-5 mm long		
<input type="radio"/> eggs	<input type="radio"/> larvae	<input type="radio"/> pupae	<input type="radio"/> adult fly	<input type="radio"/> all stages	<input type="radio"/> don't know

**As fruit fly can affect you, your neighbour or your community, the actions you take can have an impact in the control of fruit fly population. Even if you don't have fruit trees on your property you may participate in fruit fly control to reduce fruit fly in your area.**

- The objective of control methods is to reduce the current fruit fly population
- Methods to control fruit fly include trapping, baiting and disposing of fruit waste and infested fruit.

8. Select the fruit fly stage(s) targeted by each of the following control methods:

Control Methods	Developmental stages				Don't know
	eggs	larvae	pupae	adult	
baiting	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
trapping	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
spraying	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
burying infested fruit	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
removing fallen fruit	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

9. The following represent your intentions to control fruit fly on your property:

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
I intend to control fruit fly on my property in the next six months.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**10. How effective are each of the following methods to reduce fruit fly numbers?**

	Very ineffective	Ineffective	Neither effective nor ineffective	Effective	Very effective	Don't know
Removing fallen fruit	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Composting infested fruit	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Burying infested fruit	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Baiting fruit trees	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Applying chemical sprays	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Please select the best representation of your opinion regarding fruit fly control.

**11. Overall I think that controlling fruit fly on properties is:**

	Neutral ←-----→								
demanding	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	easy
complicated	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	simple
worthless	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	rewarding
effective	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	ineffective
affordable	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	expensive
harmful to health	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	safe to health

Remember: Even if you don't have fruit trees on your property you may participate in fruit fly control.

**12. How much would your decisions to control fruit fly on your property depend on the following control treatment conditions?**

	Not at all	Very little	Somewhat	Quite a lot	Very much
speed of results	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
complexity	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
effectiveness	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
cost	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
health safety	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

13. How confident would you feel to control fruit fly if the treatments are:					
	Not at all confident	Not very confident	Somewhat confident	Very confident	Extremely confident
slow	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
complicated	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
irregular in results	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
expensive	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
toxic	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Remember: Even if you don't have fruit trees on your property you may participate in fruit fly control.

14. Select your level of agreement with the following statements:						
	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree	Don't know
People who are important to me think that I should apply fruit fly control treatments on my property.						
14a	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I am confident that I can control fruit fly if I want to.						
14b	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I am not confident with the use of baits to control fruit fly.						
14c	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
At least one of my neighbours believes everybody should control fruit fly on their property.						
14d	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I believe that controlling fruit fly on my property protects other people's fruit trees.						
14e	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I am less likely to control fruit fly if treatments take too long to provide results.						
14f	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
My neighbour's fruit trees are not affected by what I do on my property.						
14g	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Spraying chemicals offers a quick solution to eliminate fruit fly on properties.						
14h	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Controlling fruit fly requires a lot of time and help.						
14i	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I am less likely to implement control treatments if these are expensive.						
14j	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Controlling fruit fly on my property has little impact on the fruit industry in Western Australia.						
14k	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

15. Please indicate your level of agreement with the following statements:						
	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree	Don't know
If I spray chemicals, I will be able to control fruit fly.						
15a	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
If fruit fly control treatments are expensive I will not use them.						
15b	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I will apply control treatments if they eliminate fruit fly immediately.						
15c	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
If I use a single type of control treatment, I will be able to control fruit fly.						
15d	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Controlling fruit fly on my property will help eliminate fruit fly from my area/ region.						
15e	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
My neighbour will have less fruit fly on their property if I control fruit fly on mine.						
15f	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

*Remember: Even if you don't have fruit trees on your property you may participate in fruit fly control.*

16. If the following person asked you to implement fruit fly control treatments on your property to help control fruit fly on their property, indicate your likelihood to take action:					
	Very unlikely	Unlikely	Undecided	Likely	Very likely
Fruit grower					
16a	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Neighbour					
16b	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

17. Select your level of agreement regarding fruit fly control.						
	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree	Don't know
Organic chemicals are a safe choice to control fruit fly on properties.						
17a	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Controlling fruit fly on my property will reduce the problems to the fruit industry in Western Australia.						
17b	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cleaning properties is a demanding option for control of fruit fly.						
17c	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

18. Please indicate desirability of the following fruit fly control issues:					
	Highly undesirable	Undesirable	No opinion	Desirable	Highly desirable
Producing fruit without chemical residues					
18a	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Eliminating fruit fly from my area/ region					
18b	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Using expensive control methods					
18c	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Eliminating fruit flies quickly					
18d	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Having extra help and time to apply control treatments					
18e	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Applying several methods simultaneously					
18f	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

19. Please indicate your level of agreement with the following statements:						
	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree	Don't know
Most fruit growers believe everybody should control fruit fly on their property.						
19a	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I am not confident with the use of traditional pesticides because of possible effects on health.						
19b	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
It is possible to get good results with control treatments that are easy to use.						
19c	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
The cost of control treatments has an impact on how fruit fly control is done on properties.						
19d	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
People are less likely to control fruit fly with baiting if it attracts more fruit flies onto their property.						
19e	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Traditional pesticides are a safe choice to control fruit fly on properties.						
19f	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Please select the one statement that best reflects your experience:

**20. In general, my experience with fruit fly control could be expressed as follows:**

- ☐ I tried control treatments but they did not work. (I didn't get rid of fruit fly.)
- ☐ I tried control treatments but they only partially worked. (I have less fruit fly after treatments.)
- ☐ Control treatments worked for me. (I no longer have fruit fly.)
- ☐ I have never applied any treatments.

**21. Order from 1 to 7 to indicate the influence the following may have in your decision to control fruit fly:**

(1 = the most influence; 7 = the least influence)

- ☐ Producing food that is safe.
- ☐ Elimination of fruit flies is permanent.
- ☐ Results of control are immediate.
- ☐ Minimal labour is required to control fruit fly.
- ☐ Control methods are simple.
- ☐ Control methods are affordable.
- ☐ Fewer types of treatments are needed.

Complete this question only if you have applied control treatments on your property to control fruit fly in the last 12 months

**22. Approximately, how much time and money have you spent in the last 12 months in fruit fly control activities on your property?**

	Time (hours per year)	Money	
		Treatments (\$ per year)	Labour (\$ per year)
baiting			
trapping			
spraying			
cleaning unwanted or fruit fly infested fruit			

*If you have any comments you want to add, please do so here.*

**Thank you for participating in this survey.  
Add your contact details if you want to enter the prize draw.**

## How can we better communicate among fruit fly fans?

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### Abstract

Networking and communication services available to the fruit fly community are the [TEPHRITID WORKERS DATABASE \(TWD\)](#), [Directory of Mass Rearing Facilities \(DIR-SIT\)](#), the [International Database on Insect Disinfestation and Sterilization \(IDIDAS\)](#), [FACEBOOK](#) page of TWD, [FRUIT FLY NEWS](#) newsletter ([FFN](#)), [TEAM](#) newsletter ([TEAM NL](#)), and [IPC](#) newsletter ([IPC NL](#)). These tools help members share research findings, experiences and expertise, post requests to fruit fly experts, inform colleagues about job opportunities or publish alerts and press releases.

TWD is a web-based database collecting and sharing information on tephritid fruit flies, which was recently upgraded to adopt and adapt to the new changes in information technology. Any fruit fly worker is welcome to [register](#) and be part of this community. During the time TWD was off, we worked hard to build a better information service web site — adding new tools, and protecting your privacy. The new version provides a robust security system of users' accounts managed through the *Nucleus* service, a new style of members' personal profile, recent literature, manuals, and guidelines. Newsletters and events are presented in a new and friendly look. A photo gallery was built-in for storing photos of the most important fruit fly events. A forum platform is now also available for the exchange of views on topics of interest.

Do you know that Tephritid fruit fly workers like you are part of a global community of decision makers, leaders, researchers and plant protection managers that advocate fruit fly free fruit and vegetables? Currently, TWD includes 1,450 members from 107 countries, belonging to three different regional groups: Tephritid Workers of the Western Hemisphere ([TWWH](#)); Tephritid Workers of Europe, Africa, and the Middle East ([TEAM](#)); and Tephritid Workers of Asia, Australia and Oceania ([TAAO](#)).

It is also of outmost importance to keep your profile updated in order to stay in touch with the global fruit fly community and receive news on tephritids. People want to know which projects and research activities you are working on. In the end, it is your support and your participation that keeps our non-profit information services going. Every time you use TWD, take action to join an event, post news, send a document, or photos of events. You are helping to build a better web site as repository of useful information available to all to learn about Tephritidae flies, the development of innovative technologies and the area-wide integrated pest management of pest fruit flies.

**Keywords:** communication, databases, fruit fly, knowledge management, Tephritidae.



## Communication strategy and vision

The Tephritid Workers Database (TWD) (Figure1) plays a key role at the global level in the dissemination of information about tephritid fruit flies by providing current information about strategies, techniques, products and processes on the many basic and applied topics related to the area-wide integrated pest management. However, this knowledge management is not just about systems and data, but also supports the building of human capacity.



**Fig. 1.** Tephritid fruit fly workers database home page.

But, what motivates people to share their knowledge? 1) It is true that knowledge is a resource that has inherent status and provides power. Nevertheless, as we live in a shrinking world with increasing global trade, it is important to sustainably control competing pests and diseases wherever they are in order to protect our livelihoods. Insects do not recognize our political borders. By empowering and providing access to knowledge and sharing of information, we can help build trust and benefitting the whole fruit fly community. 2) In addition, one of the important components related to the delivery of this service is to provide an easily accessible and always updated directory of fruit fly workers with their special fields of expertise (for example in fruit cold treatment). 3) On the other hand, as fruit fly workers with substantial knowledge and experience continue to retire, the risks of the loss of knowledge increase and the retention and transfer of information needs to be effectively

managed.

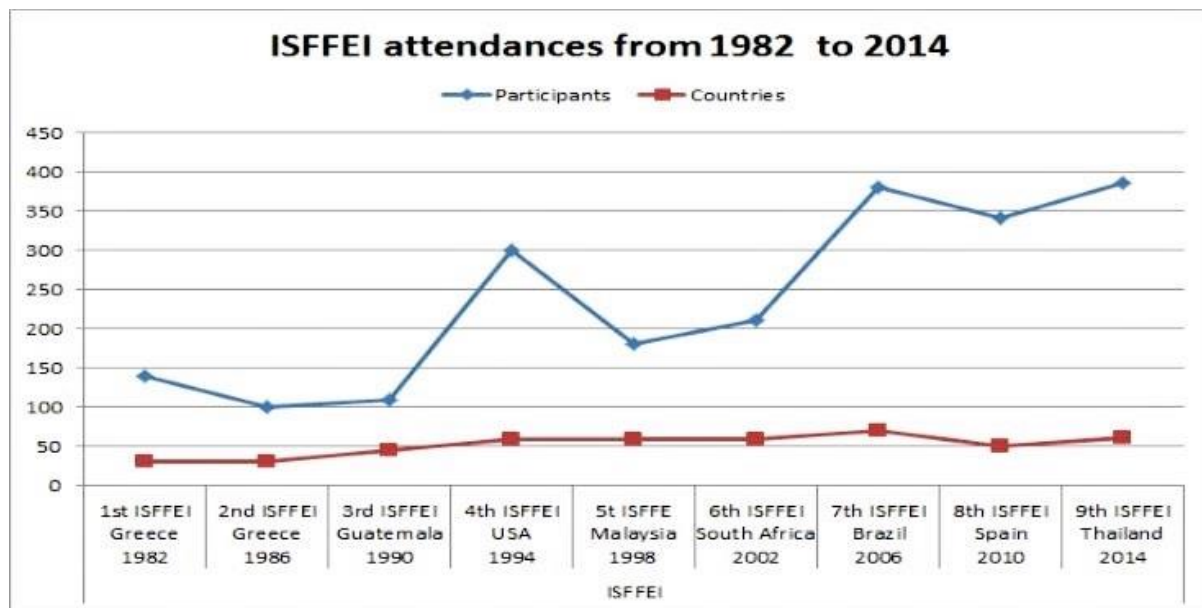
Three decades ago, pioneer fruit fly workers had initiated an excellent framework for knowledge management and information exchange. They established an International Steering Committee (SC), the International Symposium on Fruit Flies of Economic Importance (ISFFEI) held on a regular basis every four years, fruit fly working groups, and a newsletter already with a list of fruit fly workers. This article reviews the status of these services and the new developments.

### International fruit fly meetings

Initiated in 2004, TWD nowadays includes 1450 members (as of December 2014) throughout the world from 107 countries. With each region having its own specificity, for example *Anastrepha* is the main concern in Central and South America, *Rhagoletis* for the northern countries, *Bactrocera* for the Asia/Pacific and now in Africa zones in general, it was logical to have regional working groups established in order to increase collaboration at the region level in the study and management of fruit flies. These regional groups - the Tephritid Workers of the Western Hemisphere (TWWH), Tephritid Workers of Europe, Africa and the Middle East (TEAM), and Tephritid Workers of Asia, Australia and Oceania (TAAO) - have their own SC and regional meetings planned every four years and alternating with the ISFFEI. To allow members of the global fruit fly community to be able to participate in any of these regional meetings, precaution is taken by chairs of the SCs to have different venue dates (Table 1). These meetings are a good opportunity to interact, meet new members and old friends, and share the latest findings. The general trend of ISFFEI attendance shows a steady increase from the first global symposium in 1982 to the ninth symposium in 2014 (Fig.2), with the organisation reaching increasingly high standards.

**Table 1.** Planned tephritid fruit fly meetings.

	<b>TAAO</b> 1 <sup>st</sup> meeting 	<b>TEAM</b> 3 <sup>rd</sup> meeting 	<b>TWWH</b> 9 <sup>th</sup> meeting 	<b>ISFFEI</b> 10 <sup>th</sup> meeting
<b>Date</b>	15-18 August 2016	11-14 April 2016	16-21 October 2016	9-13 April 2018
<b>Venue</b>	Kuala Lumpur, Malaysia	Stellenbosch, South Africa	Buenos Aires, Argentina	Tapachula, Chiapas, Mexico



**Fig. 2.** Chart of the participants of the International Symposium on Fruit Flies of Economic Importance (ISFFEI) from 1982 to 2014.

### Fruit fly newsletters

Newsletters are a good communication tool in order to keep the community in touch and informed about the new developments at the regional and global level. Currently there are two newsletters specific to tephritid fruit flies: Fruit Fly News ([FFN](#)) and TEAM newsletter ([TEAM-NL](#)), as well as a global newsletter of the Insect Pest Control Section of the Joint FAO/IAEA Programme ([IPC-NL](#)) which include pertinent information on fruit fly applied research and control (Fig.3).

Established in 1972 by Ernst Boller and distributed annually in a paper format, FFN is quarterly distributed in electronic format, since February 2012, to 1450 members from 107 countries (Fig.4). Back issues from FFN 1 to FFN 29 are available on the TWD site. TEAM-NL was established in 2005 and is distributed twice a year to members from the region. IPCS-NL was established in the 1960's and is currently distributed twice a year (January, July).

### TWD News and Facebook (FB)

While these meetings and newsletters are important for keeping the community updated, continuous news feed is also an important tool for reporting the rapid flow of information in the fruit fly field. In a less informal way, TWD News (Fig.1) and the Facebook page of TWD (Fig.5) can fill this task by posting alerts and news on outbreaks of the most important events in a nearly instantaneous manner. In addition, comments can be easily posted on the Facebook wall and may trigger stimulating discussion on a specific topic.



Fig. 3. Front page of FFN, TEAM and IPCS-NL.

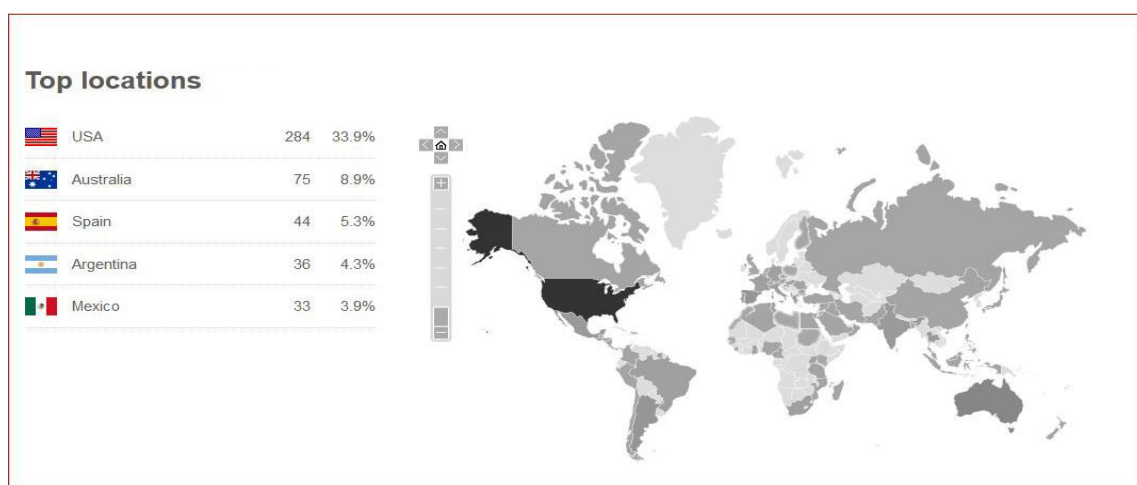
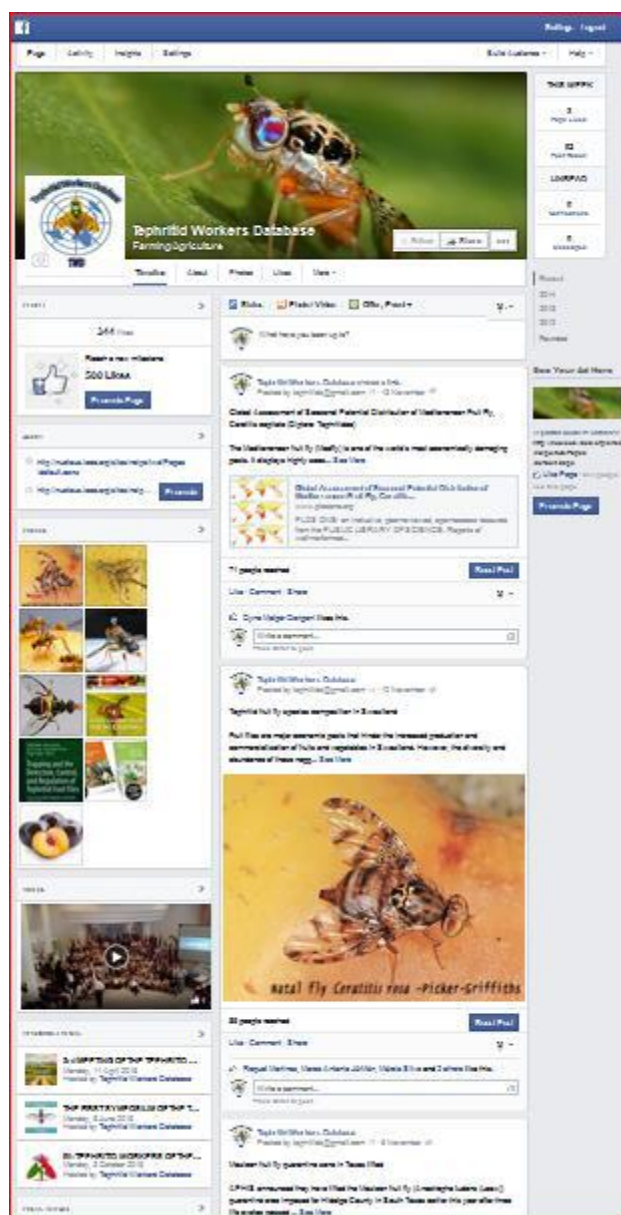


Fig. 4. Global distribution to 1450 members from 107 countries. Fig.shows countries of members

having opened the FFN29, sent on October 29, 2014.



**Fig. 5.** Facebook page of TWD.

## Conclusion

The aim of our work is to facilitate the communication among the tephritid fruit fly community. Various communication tools have been set up to suit different needs. In the end, it is your involvement and participation that help to spread the information in these networks and above all to educate the world about the importance of the fruit flies and how to deal with these pests in a more environment-friendly and therefore more sustainable way. Finally, we would like you to keep your profile in TWD updated in order to stay connected.

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## Annex

[IAEA.org](#)
[NUCLEUS](#)


**IAEA**

**NAIPC**
Nuclear Applications for Insect Pest Control



[Register](#)
[Sign In](#)

[TWD](#)
[DIR-SIT](#)
[IDIAS](#)
[IDGT](#)

Organization

- Joint FAO/IAEA Programme
- Insect Pest Control Section
- Insect Pest Control Laboratory

Tephritid Fruit Fly Steering Committees

Regional groups

News

Newsletters

International-Fruit-Fly-Symposia

Entomology Events

Search\_Publication

Operational projects

Resources

Member Areas

Photo Gallery

### Member Information

#### Member Information

##### How to Become a Member

1. Register on [NUCLEUS](#) and fill out the simple form. NUCLEUS is the IAEA Nuclear Knowledge and Information portal.
2. An account activation message will be sent to your email address.
3. Please follow the "Activate Account" link to complete your account creation and gain access to the TWD site's resources under [NA-IPC](#).
4. Please inform the administrator of your NUCLEUS accounts.
5. If you already have a NUCLEUS account, you will just need to inform the administrators of your account.

##### What you can do as a Member

1. You can read all the available information on the [TWD](#) page.
2. You can take part in the discussion forum.
3. As a member you will have access to our database with over 1200 members from across the world, thus being able to establish contacts and stay in touch with the community of tephritid workers.

##### What you can do as a Non-Member

1. Non-Members can read all the available information

**[JOIN TODAY](#)**

## **Know thy neighbour: Turning weakest links into Mediterranean fruit fly warriors to achieve Area Wide Management**

**Isabel Arevalo-Vigne<sup>1,3</sup>, Ben White<sup>1,3</sup> & Nancy Longnecker<sup>2,3</sup>**

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### **Abstract**

*Introduction:* Area-wide management (AWM) is an integrated approach in which pests are managed using an organised and coordinated control of target pest populations over large areas rather than using an individual property approach. The effectiveness of controlling Mediterranean fruit fly (medfly), *Ceratitis capitata*, can be jeopardised by the degree of cooperation of growers and the general public. Uncoordinated or even partial actions by individuals are the weakest links in an AWM scheme as these individuals can undermine the actions of those complying with the scheme. The push for the use of safer products has led to the ban of organophosphate cover sprays to control medfly in Western Australia. This has created doubts among fruit growers on the effectiveness of current methods with some unable to observe ‘fast’ and permanent results to the medfly problem in their area, questioning the validity of the AWM approach.

*Methods:* Under the framework of the Theory of Planned Behaviour we intended to evaluate direct and indirect measures of predictor variables of people’s participation in fruit fly control on commercial properties, small landholders and backyards. Surveys of citrus and stone fruit growers and the public in urban, peri-urban and rural areas in the southwest of Western Australia were designed to measure: a) people’s attitude to implementing fruit fly control methods; b) their perceived social pressure to perform control methods; and c) people’s perceived effectiveness in implementing control methods.

*Results:* From a sample of 605 people from Western Australia, 498 indicated they were growing fruit trees on their properties. From this group, only 49% applied control methods for medfly and only 8.9% of them expressed 100% satisfaction with results provided by control treatments. Achieving community engagement is a complex matter, and there are indications that a person’s likelihood to participate in or adopt control methods depends just as much on their understanding of the ecological issues involved as on other factors which may diminish their motivation (e.g. financial costs, time required). When we explained the benefits of controlling medfly collectively, 65% of survey respondents agreed to implement control methods in the next six months - including those without fruit trees.

*Discussion:* Negative perceptions of the effectiveness of safer control methods and costs

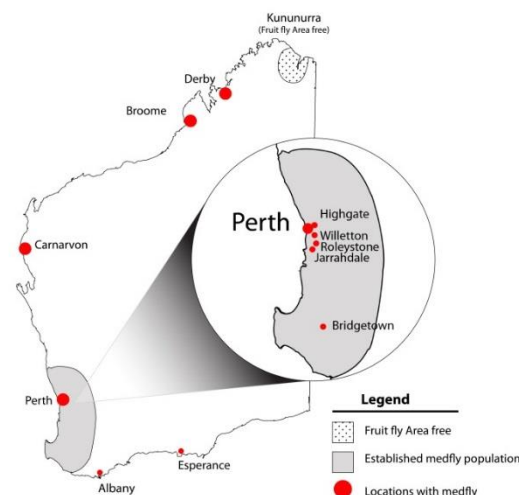


associated with control and property sanitation are added to growers' and household owners' frustration with the lack of participation of members of their own communities. Removing the weakest links or, better yet, turning them into active medfly warriors requires an understanding of people's perceptions and beliefs regarding the adoption of medfly control methods and the role of their participation.

**Keywords:** area wide management, Mediterranean fruit fly, medfly, science communication.

## Introduction

The Mediterranean fruit fly (medfly), *Ceratitis capitata*, is an exotic pest to Australia. It was introduced into Western Australia (WA, Perth) in 1895 (Sproul et al., 2002), probably from cargo originating from South Africa and gradually expanded into fruit production areas between Perth and Bunbury. Currently, medfly is distributed in the south west of WA (Fig.1) and is found from Esperance through to Carnarvon. It is also found in the north of the state in Broome and Derby but it is absent from the Ord River Irrigation Area (Kununurra), which has fruit fly free status (DAFWA, 2002; 2013). In addition to commercial orchards, medfly is found on properties in urban, peri-urban (neither urban nor rural) and rural areas where fruit species are grown. Like other fruit flies, medfly takes advantage of the presence of irrigated environments such as backyards (Bateman, 1972; NSW DPI, 2006) to survive between seasons. Due to the climatic conditions of WA, the increase of irrigated areas (DoW, 2005) and the presence of numerous varieties of fruit tree species, medfly can be found all year round. The presence of fruit trees in backyards has increased the pressure of fruit fly populations on commercial fruit growing areas, particularly to those properties in close proximity to urban dwellings.



**Fig. 1.** Distribution map of *Ceratitis capitata* in Western Australia. An online survey was distributed around the State while direct surveys were obtained from randomly selected households in Willetton, Roleystone, Jarrahdale, Highgate and Bridgetown (inset).

Historically, the control of this pest is affected by the development of technology and policy

initiatives. Between 1950 and 1975, a fruit baiting scheme was operated in both urban and rural properties resulting in the suppression of medfly populations to acceptable levels (Sproul et al., 2002). The scheme was considered extremely effective due to the relative isolation of orchards and the use of organophosphate insecticides, such as dimethoate and fenthion, in baits or cover sprays. In the late 70s, despite the success of the technology and the increase of baiting schemes in the state, the program became unpopular because it required increasing funding and cooperation between orchardists and householders (DAFWA, 2002). Over time the decline in value of the horticultural production has led to the fragmentation of horticultural areas and changes in land use in WA. Hobby farms, neglected orchards and urban encroachment have resulted in the increase of fruit fly populations and this has imposed significant costs on fruit industries in the south west of Western Australia (DAFWA, 1998; 2014).

As a result of the increase of medfly populations in and around commercial orchards, fruit growers became more reliant on organophosphate cover sprays to control medfly and to reduce the continuous losses in fruit production. However, the Australian Pesticides and Veterinary Medicines Authority (APVMA, 2011; 2013) suspended the use of dimethoate in 2011, and imposed severe restrictions to the use of fenthion in 2013. Both decisions were made after extensive reviews on the risks posed by insecticide residues in fruit to human health and the environment. Efforts to reduce fruit fly numbers with other means of control has increased as the use of fenthion was phased out in 2015, leading growers and policy makers to explore alternative control measures, including AWM.

#### *Area Wide Management in Western Australia*

The dilemma posed by the removal of effective chemical control treatments is addressed worldwide with Area Wide Management (AWM) programs. AWM is an integrated pest management approach supported by coordinated, sustainable and preventative strategies that targets the entire pest population within a delimited geographical area. This approach requires all stakeholders to participate consistently in fruit fly control all year round (Hendrichs et al., 2007). The AWM strategies are designed to include practices that control pests while reducing the use of pesticides (FAO, 2014a; 2014b). AWM tends to reduce fruit fly populations gradually over time by integrating different control methods. This has created doubts among fruit growers on the effectiveness of AWM methods with some growers uncertain about the effectiveness of AWM at controlling outbreaks and about its long term viability.

Low participation rates from households in urban and peri-urban properties in controlling medfly are an increasing threat to fruit industries in Western Australia. To be successful, medfly control under AWM requires complete compliance by stakeholders with control methods. However, achieving AWM is jeopardised by poor cooperation between commercial fruit growers and the public, and by poor management practices on individual properties. Improving people's participation in the control of medfly in support of the fruit industry in WA (Jessup et al., 2007) requires understanding people's motivations and constraints to adopt

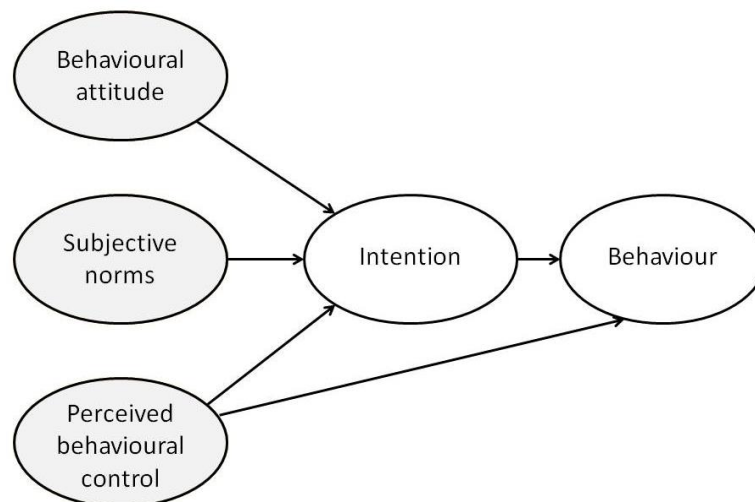
and sustain medfly control on their own properties. Understanding uncooperative behaviour and patch adoption of effective control methods is the key to designing successful AWM schemes.

### *The Theory of Planned Behaviour*

Studies on integrated pest management programs in farming situations (Heong & Escalada, 1998; Escalada & Heong, 2010; Mzoughi, 2011; West & Cisse, 2014) indicate that successful schemes address the drivers that determine program adoption.

Ajzen (1985; 1991) developed the Theory of Planned Behaviour, a theory that explains the links between attitudes, beliefs and behaviour. This theoretical model proposes that the prediction of the occurrence of a particular behaviour is based on the measurements of psychological constructs (Mathieson, 1991) that define an individual's intention to perform a particular behaviour (Fig.2).

Attitudes toward a behaviour, subjective norms, and perceived behavioural control together shape an individual's behavioural intentions and behaviours (Ajzen, 2002; 2011). Attitudes consist of a collection of beliefs and feelings regarding a particular outcome and the behaviour that led to that outcome. Subjective norms are made by an individual's perception of what others think about the individual performing a particular behaviour. Social pressure comes from relatives, friends or from social groups (such as neighbours, associations) whose opinions the individual values. Perceived behavioural control is made of an individual's self-analysis of its own efficacies to perform behaviour with the elements considered necessary for the outcome expected from such behaviour.



**Fig. 2.** Schematic representation of the Theory of Planned Behaviour (Ajzen, 1985).

Analysis of the variables involved in the behavioural constructs leading to the formation of a behavioural intention to control medfly can help design strategies that enable the adoption of positive behaviours towards the implementation of AWM to control medfly in WA. The aim

of this study was to evaluate the factors involved in an individuals' formation of intention to control medfly on their property, and which could be hindering the adoption and performance of medfly control in WA. Based on the Theory of Planned Behaviour, a statewide survey was prepared to collect information regarding people's attitudes and perceptions around medfly control methods and their understanding of the scientific facts regarding medfly control. In particular we focused on determining attitudes towards implementing fruit fly control methods; the perceived social pressure to perform control methods; and the perceived self-control over the implementation of control methods.

## **Material and Methods**

### *Constructs*

In order to use the Theory of Planned Behaviour in the adoption of control measures against medfly, we need to state the behaviour in terms of target, action, context and time (TACT), as these are requisites for attitude measurement (Ajzen & Fishbein, 1980). According to this, the more specific a question to fit TACT requirements, the better the measurement of the behavioural-intention-behaviour relationship. In this research the stated behaviour is 'the ongoing control of fruit fly on properties'. Constructs for the survey were drawn from interviews with entomologists and fruit fly project managers from the Department of Agriculture and Food Western Australia (DAFWA), from fruit growers, and from survey reports from the Hills Orchard Improvement Group.

The attitude constructs being measured were factors that individuals perceived as outcomes from the control of medfly on properties and that were influenced by changes in policies, funding and technology advancement:

- Demand – regarding the efforts necessary for the implementation of control measures. The full suite of control measures proposed under area-wide management requires more time and money than with the application of cover sprays. In general, backyards fruit growers do not follow management protocols as control is done in an ad-hoc manner.
- Complexity – of control requirements. Control measures have to be implemented accordingly to season, weather patterns, and types of host plants. These include baiting, trapping, hygiene around properties and the disposal of infested fruit.
- Worth – perceived satisfaction value and or reward as a result of control. Opinions depend on how much satisfaction (economic or personal) fruit growers get from the control activity.
- Effectiveness – Area-wide management is being promoted by the State Government as an integrated approach to control (but not to eradicate) fruit fly. On the other hand fruit growers oppose the removal of cover sprays because it takes away a quick and effective control method.

- Cost – State funding for the control of fruit fly in the southwest of WA is no longer available so the fruit industry should fund control as part of their business. However the fruit industry indicates that control costs affect the sustainability of their businesses due to severe infestations.
- Safety – Organophosphates are removed because of health risks, however, commercial growers do follow withholding period protocols.

### *Data collection*

The survey (Arevalo-Vigne et al., 2016) collected participants' demographic information: postcode, gender (male/female), location (where fruit is grown: house/orchard/other), and age. Age was grouped into five intervals similar to those used by the Australia Bureau of Statistics. The survey asked questions regarding perceptions of the demands and results of using the control techniques; how control characteristics influence decisions and confidence to control fruit flies, as well as perceptions of safety. It also enquired about social influences; and the potential impact of control at local, regional and state levels.

Direct attitudinal questions were on a seven point scale (1 to 7) (Annex) to measure attitude variation towards medfly control. Endpoints of the scale addressed the perceptions of demand, efficiency, effectiveness, cost, worth and safety derived from the control outcomes. On the other hand, the response choices for perception questions were given on a five-point Likert-type scale (Annex 2). Endpoints of the scale depended on a) levels of agreement with statements, including intention and confidence = strongly disagree/strongly agree; b) desirability of control issues = highly undesirable/highly desirable; and c) influence from others = highly likely/very unlikely.

The survey included a question to assess an individual's overall experience with fruit fly control – complete success, partial success, complete failure or never implemented in the past. Additionally the survey also collected comments regarding people's views on issues affecting the medfly problem.

The survey was conducted between November 2013 and March 2014. It was distributed in two formats: online (Internet) and hard copies. Invitations to participate were sent via email for re-distribution to members and networks from community garden groups, ethnic and cultural associations, individuals, fruit industry groups and gardening social media and news media networks. Additionally, hard copies of the survey were delivered to and collected from home properties from randomly selected street blocks in the suburbs of Willetton, Jarrahdale, Highgate and Bridgetown.

### *Data Analysis*

Data was analysed to measure the association of independent variables identified as attitudes

(the control was perceived as worth, complex, efficient, effective, demanding, or expensive), subjective norms (the influence of neighbours, fruit growers or others) and perceived behavioural controls (confidence to be able to control if control is seen as slow, irregular, complex, toxic or expensive) in the intention to control medfly as the dependent variable. The analysis was applied to groups within the sample, such as property type, occurrence of fruit growing activity, occurrence of fruit fly control by groups within the sample growing fruit trees and involved in active control. Statistical analysis was done based on valid cases using STATA (13.1) software. However, not all questions were answered so missing data exists. STATA handles missing data by omitting the missing values using "listwise deletion" and computing the prediction model on non-missing cases. Regression analysis were used to investigate the independent contribution of different variables to potential predictors of the following outcomes: 1) Implementing medfly control of properties to help others is influenced by a high regard to help the state; 2) General experience towards control is highly influenced by the effectiveness of control on properties; 3) People's intention to control is influenced by the cost of the treatments; 4) Fruit tree owners fail to control because the control treatment is complicated; and 5) People's confidence in control is influenced by all the control characteristics.

## Results and discussion

The results indicate that despite the large proportion of respondents that grow fruit (Table 1), only 57% of them control fruit fly.

**Table 1.** Proportion of related fruit growing activities a) by fruit growers' involvement in control of *Ceratitis capitata*; and b) according to the location where fruit trees are grown.

(a) Proportion of activities		Total*	%*
Grow fruit trees		494	83%
Control medfly		274	55%
Don't control medfly		210	43%
(missing data)		10	2%
(b) Proportion of activities by location	House	Orchard	Other**
Grow fruit trees	81%	18%	1%
Control medfly	78%	21%	1%
Don't control medfly	86%	13%	1%

\* calculated on valid cases; \*\* community gardens, apartment.

### *Fruit fly control and growers' age*

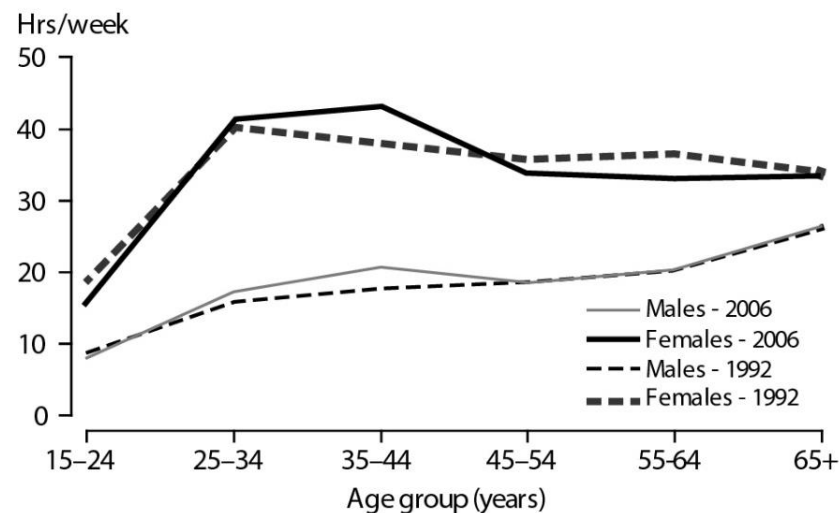
Comparison of responses from fruit growers among each age category (Table 2) indicated that the decision to apply control measures increased with age and that people under 35 years old tended not to

participate.

**Table 2.** Distribution of fruit grower responses (%) by age group.

	Age group					
	< 25	25 -34	35 -44	45 -54	55 -65	> 65
Grow fruit	60%	65%	82%	86%	86%	91%
control medfly	17%	36%	51%	56%	59%	71%
house	100%	95%	86%	78%	73%	85%
orchard	0%	5%	14%	19%	27%	15%

This is consistent with the Australia Bureau of Statistic's report (2009) on how age groups spend time on household work (Fig.3). For example people aged under 25 years old are less likely to engage in domestic activities. Demographics studies of fruit growers are important as age determines how active people are or how capable and confident people feel about undertaking tasks (Beaujot & Liu, 2005; Grusec et al., 1996).



(a) Average hours per week by all persons for primary activities

Source: ABS Time Use Survey, 1992 and 2006

**Fig. 3.** Comparison of Australian's time use patterns of household work in 1999 and 2006 (Source: Australian Bureau of Statistics 2009). Time involvement in domestic activities can vary throughout different stages of life. For example, people under 34 years old are less involved in housework than other age categories.

### *People's intention to undertake control on their properties*

The survey included direct and indirect measurements of the behavioural constructs to control fruit fly. The intention to perform a given behaviour was expressed as the interaction of direct measurements:

$$\text{Intention} = \text{Attitudes} + \text{Subjective Norm} + \text{Perceived Behavioural Control}$$

The regression of the variables involved in the intention to control fruit fly (Table 3) showed that the attitude 'fruit fly control is safe for health' was highly significant for the whole sample. This result indicates that the use of organophosphate cover sprays in fruit produce is an issue for the whole population.

Trends of Australian agriculture have shown that industry and regulators have been adapting to the increasing pressures of international markets for the production of food without chemical residues (Radcliffe, 2002). Reduction of pesticides in agriculture means prioritising the health of consumers and the environment, requiring the implementation of technologies that are proven safe. The results regarding attitudes towards the safe use of control methods for fruit fly matches the increasing demand from consumers for 'more organic' produce.

Results from Table 3 show that for the whole sample the attitude towards safety provided by the control as well as social pressure has an influence on the intention to apply control on their properties. However, this was not observed in the group that grow fruit trees and only the attitudes of the worth and effectiveness of the control were significant in the sub-group that apply control against medfly on their properties.

**Table 3.** Regression analysis of individual's intention to apply control on their properties under the Theory of Applied Behaviour model.

Psychological constructs	Whole sample		Fruit growers			
			Apply control		Don't apply control	
Attitudes	Coef	P	Coef.	P	Coef.	P
Demand	-0.013	0.755	-0.039	0.172	0.049	0.556
Complexity	-0.022	0.625	-0.002	0.938	-0.096	0.298
Worth	0.089	0.039	0.084	0.008	0.123	0.126
Effectiveness	-0.045	0.207	-0.073	0.008	-0.043	0.473
Cost	0.028	0.382	-0.017	0.433	0.023	0.733
Safety	0.127	< 0.001	0.033	0.213	0.094	0.152
Subjective Norm	0.330	< 0.0001	0.030	0.481	0.092	0.290
Perceived Behavioural Control	-0.091	0.100	-0.005	0.905	-0.017	0.864



*Implementing medfly control is influenced by a high regard to help the State.*

In 2002 DAFWA launched its biosecurity program to protect the state from the entry and spread of pests and diseases. With the adoption of the slogan ‘Biosecurity is everyone’s responsibility’, DAFWA brought attention to the impact everyone can have in the biosecurity of agriculture and emphasised the importance of wide participation to protect agricultural industries (Delane & Lloyd, 2002).

Since then, DAFWA has worked to increase landholder, industry and community awareness of pest and diseases threatening agriculture and which can have a negative impact on the economy of the state. By participating actively in monitoring and control of pests on their properties the public will be helping the agricultural industry.

In the case of medfly and the fruit industry, this approach assumes that people regard the fruit industry highly, and that supporting the state is a strong motivator in encouraging people’s participation in biosecurity measures to reduce the fruit fly problem.

However, contrary to the above assumption, Table 4 shows that in general, people were more prone to believe that controlling fruit flies on their property will help their region. This regional consciousness is explained by an individual’s identification with a place, with a ‘sense of home’ constructed on personal, social and cultural meanings. ‘In either case, place identities affiliate the self with significant locals, bringing a sense of belonging and order to one’s socio-spatial world’ (Cuba & Hummon, 1993).

**Table 4.** Multivariate linear regression analysis – correlates of behavioural beliefs that ‘groups benefit of control on properties’ with ‘control to protect other people’s fruit trees’ as dependent variable.

Belief	Grow fruit trees		
	All (N=533)	Apply control	Don’t apply control
Helps neighbours	0.361	0.033	0.060
Helps region	< 0.0001	< 0.0001	< 0.0001
Helps WA fruit industry	0.584	0.006	0.687

*People’s intention to control is influenced by the cost of the treatments*

A regression analysis was done to understand the individual effects each of the identified attitudes had on the intention to control fruit fly, particularly to measure the effect of costs in implement fruit fly control.

Individual needs drive people’s purchasing behaviours and decisions. In most cases, price plays an important role in their buying decision. An individual’s decision to purchase certain products over others is often limited by the availability of monetary resources, meaning that sometimes people need to choose some things and reject others (Kenesei & Todd, 2003).

One of the arguments from commercial fruit growers towards the implementation of area-wide management protocols is that the cost of control methods will not make fruit production cost effective. However, from the results presented in Table 5, the only factors having a significant influence to orchardists are the attitudes of the control being worth doing and safe to implement. Because this group includes both commercial growers and small landholders the results cannot exclude the possibility of changes to the significance of the cost attitude in their intention to control behaviour in commercial growers when the perception of reward reduces.

Conversations with backyard fruit growers revealed that this group seems not to have a particular preference for either low cost control strategies with homemade traps or buying commercial products to control fruit fly which agrees with the results in Table 5 in which the cost of the control scheme/strategy/measure/technique has no influence in their intention to control fruit fly.

**Table 5.** Multivariate linear regression analysis of the whole sample grouped by location, and fruit growers– correlates of attitudes towards the control of *Ceratitis capitata* with intention as dependent variable.

Attitudes	Grow fruit (N=459)		Don't grow fruit (N=86)		Location			
	Coef.	P	Coef.	P	house		orchard	
Demand	-0.049	0.226	0.156	0.126	-0.025	0.580	-0.167	0.093
Complexity	-0.052	0.248	-0.001	0.988	-0.065	0.187	-0.033	0.770
Worth	0.188	<0.0001	0.133	0.115	0.184	<0.0001	0.256	0.015
Effectiveness	0.024	0.501	-0.002	0.985	0.021	0.599	-0.017	0.861
Cost	0.001	0.973	-0.206	0.021	0.000	0.997	0.064	0.434
Safety	0.162	<0.0001	0.041	0.620	0.172	<0.0001	0.113	0.206

Attitudes based on costs only seem to influence those individuals that currently do not grow fruit. The implication of this result is that in the eventuality non-fruit growers decide to grow fruit or move to a property which already has fruit trees, these ‘forced’ new fruit growers will have problems engaging in control behaviour based on control cost issues. Alternatively, they can change their mind once they are more involved in the situation.

The results of the analysis also reported that the attitudes towards worth and safety were significant in fruit growers intention to control medfly.

The attitude that controlling fruit fly is worthy seems to influence fruit growers’ intention to control medfly; and particularly responsible for householder’s not controlling fruit fly on their properties (Table 6). This last group is also influenced by the attitudes towards the complexity

and safety of the control. The interaction of these three attitudes could explain the low uptake of control by the community.

A probable explanation why safety was not a significant factor is that for more than two years householders have not been able to use organophosphates for fruit production. This, plus the increasing trend of organic approaches with biocontrol, exclusion nets and homemade baits and traps may have created a concept that control in itself is already safe.

A participant indicated ‘I am considering pulling out all of my fruit trees as I cannot safely and effectively control fruit fly. I am willing to risk lebaycid again, as I think that perhaps that was the most effective and easiest treatment of all (despite the so called possible harmful effects).’

This statement highlights an important issue for the future of fruit production without organophosphates. Fruit growers, in particular those growing fruit in orchards, may try to find other products to deal with the problem in a quick and effective way without considering the impacts on human health and the environment.

Another respondent indicated “The fauna I have in and under my fruit trees does all the ‘work’ and maintains control for me. I would not exchange this for labour intensive practices or chemicals, ever.” This type of approach demonstrates that people are resorting to methods to reduce the labour required under an integrated pest management approach, with possible serious consequences to the fruit industry.

**Table 6.** Multivariate linear regression analysis of fruit growers group that apply control or not and according to type of property – correlates of attitudes towards medfly control with intention as dependent variable.

Attitude towards control	Apply control				Don't apply control			
	household		orchard		household		orchard	
	Coef.	P	Coef.	P	Coef.	P	Coef.	P
Demand	-0.064	0.098	-0.006	0.894	0.103	0.125	-0.090	0.863
Complexity	0.002	0.962	-0.040	0.454	-0.187	0.011	0.145	0.780
Worth	0.087	0.023	0.160	0.002	0.219	0.001	0.156	0.670
Effectiveness	0.039	0.255	0.068	0.174	0.083	0.131	-0.230	0.377
Cost	0.000	1.000	0.059	0.185	-0.007	0.903	0.023	0.912
Safety	0.027	0.424	0.010	0.868	0.134	0.019	-0.102	0.694

In summary, financial cost does not influence fruit growers' intention to control fruit fly. Instead, the results indicate that the attitude towards the worth or reward perceived from the control behaviour is probably responsible of the overall intention to embark in fruit fly control. The attitudinal worth variable was significantly stronger for the group of respondents

growing fruit trees and in particularly for those growing fruit trees in household properties and backyards.

*People's confidence in control is influenced by the effectiveness of the control characteristics*

“As a fruit grower all we want is an effective one fits all solution. We had that with fenthion and that was taken away from us. Fruit fly control now costs us five times more and is four times less effective. Governments and decision makers highly underestimate how high the fruit fly population is in the Perth hills districts. Other fruit fly control measures have so far proven ineffective” (comment from MedflyMotivations survey 2014).

The perception of control being complex is probably influencing fruit growers' self-assessment of their capabilities and acting as a deterrent to implementing control on their properties (Table 7).

**Table 7.** Multivariate linear regression of the influence of control characteristics in fruit growers' confidence with perceived behavioural control as a dependent variable.

Confidence to control if treatment is	Fruit growers			
	Control fruit fly		Don't control fruit fly	
	Coef.	(P)	Coef.	(P)t
Slow	0.032	0.750	-0.321	0.767
Complex	-0.033	0.768	0.343	0.007
Irregular	-0.022	0.823	0.013	0.902
Expensive	0.084	0.401	-0.191	0.097
Toxic	0.106	0.139	0.077	0.358

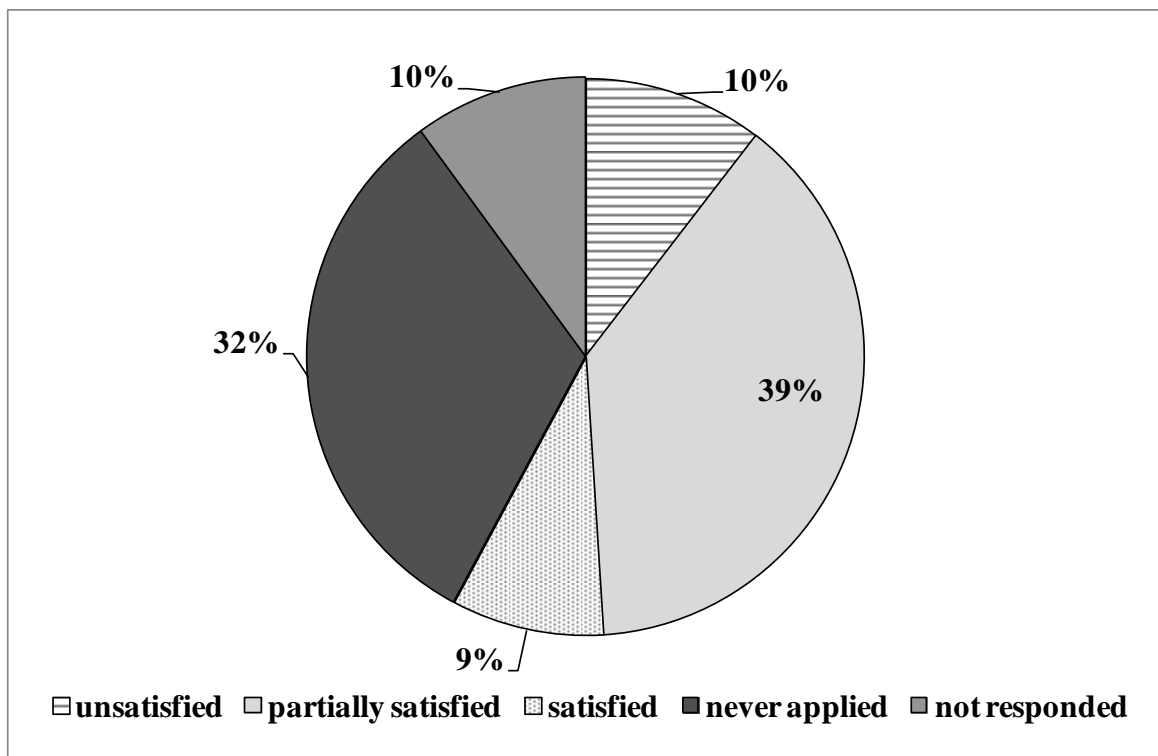
Strategies to improve people's capabilities to deal with pests and diseases are based upon increasing people's knowledge of the pest and how to use the required control methods (Price, 2001; Brown et al., 2010; Bentley et al., 2011; CABI, 2013).

*General experience towards control is highly influenced by the effectiveness of control on properties*

“I use the collection and boiling treatment of fallen fruit which is then fed to chooks to clean up any residue of fruit fly. These work most years but every now and then there is an outbreak of fruit fly so continuous setting of bait traps is to me the only way to keep the numbers low” (comment from MedflyMotivations survey, 2014).

A high proportion of people not involved in fruit fly control or not satisfied with the results of control on their properties (Fig.4) represents a high risk to the success of AWM approach because they can easily stop controlling fruit fly altogether.

It is probable that perceived confidence derived from the perception of certain characteristics of the control have an effect on the satisfaction experience. As Ajzen (1991) indicated, the degree of perceived behavioural control, which refers to the perceived ease or difficulty of performing behaviour, is assumed to reflect past experience as well as anticipated impediments and barriers.



**Fig. 4.** Distribution of fruit grower's satisfaction experience from medfly control (N=498).

The results presented in Table 8 suggest that a perception of the control being 'Complex', 'Expensive' or 'Toxic' reduces confidence in the technology while affecting the overall satisfaction experience.

These control beliefs may be based in part on past experience with fruit fly control, but they will usually also be influenced by second-hand information about the control behaviour, by the experiences of relatives, friends and acquaintances, and by other factors that increase or reduce the perceived difficulty of performing the behaviour in question.

**Table 8.** Multivariate linear regression analysis of fruit grower's self-confidence to control *Ceratitis capitata* – correlates with experience satisfaction with medfly control as dependent variable (N=498).

Experience	Coef.	Std. Err.	t	P>t	[95% Conf.Interval]	
Confidence if slow	0.057	0.062	0.920	0.359	-0.065	0.179
Confidence if complex	-0.038	0.068	-0.550	0.581	-0.171	0.096
Confidence if irregular	0.087	0.060	1.450	0.149	-0.031	0.205
Confidence if expensive	-0.078	0.062	-1.250	0.211	-0.200	0.044
Confidence if toxic	-0.023	0.045	-0.500	0.614	-0.110	0.065

## Conclusions

In this study, a measure of intention to undertake fruit fly control was tested with the Theory of Planned Behaviour to explore the elements that may influence the 'prediction' of Western Australian fruit growers' participation for the control of Mediterranean fruit fly on their own properties.

Three reasons support the use of the TPB to gain a better understanding of the factors and barriers involved in AWM for the medfly:

- 1) Due to its high predictive validity (Armitage & Conner, 2001) the theory is a useful tool to study the adoption of integrated management practices as it provides information about the factors that users consider when making their choices.
- 2) The theory is a quantitative behavioural approach that makes a distinction between voluntary and non-voluntary factors that influence individuals. As such, the theory allows evaluation of the role of these factors in intended and actual behaviour (Ajzen, 1985). The theory provides policy makers with a standardised and repeatable methodology (Burton, 2004a) that can help policy managers anticipate resistance to change due to policy formulation and implementation (Burton, 2004b).
- 3) Socio-psychological approaches are needed to explain the reasons behind selective adoption of integrated pest management practices (Ridgley & Brush, 1992; West & Cisse, 2014) and the reasons behind coordinated action with other groups (Brenner et al., 2003; McKee, 2011) as expected in AWM. Conflicts with policy instruments arise because farmers and growers differently evaluate the risks and benefits of adopting technologies against uncertainty and their potential impact on collective participation.

Under the TPB, intentions to perform a behaviour are explained in terms of attitudes toward the behaviour, subjective norms, and perceived behavioural control (Ajzen, 1985; Madden et al., 1992). Additionally, attitudes, subjective norms and control behaviour can be explained in terms of an individual collection of beliefs about the consequences of performing the behaviour, about the normative expectations of important individuals and internal evaluation of non-voluntary factors' controllability.

The results of this study have demonstrated that strategies with a ‘One size fits all’ approach to managing medfly will never succeed. The fact that individuals or certain groups may create resistance to the acceptance of certain policies, constitutes the weakest link in the problem-solution relationship and needs to be considered from a socio-psychological perspective when formulating and implementing AWM strategies.

As there are several influences on the intention to practice control of fruit fly on properties, strategies should be adapted for each particular group to increase the participation of commercial and backyard fruit growers in coordinated control, as required in AWM of medfly.

Strategies promoting the benefit of a region will be accepted better than those promoting helping the neighbour or the whole of the industry in WA.

People’s attitudes regarding fruit fly control varies within each group. Despite the general perception that cost is an issue in implementing control for medfly, fruit growers are more concerned about the fact that the efforts invested in control are worth doing.

Current trends in pest management are the adoption of softer pesticides and integrated pest management. However, the incentives to control pesticide residues in domestically consumed foods have not been as strong as for export markets. This is an issue that needs to be taken into account in developing a strategy for commercial fruit growers, as the results of this research confirm that this group is not concerned about health issues.

Being capable of dealing with fruit fly requires that the individual is confident to undertake the control. The perceived complexity of the control treatments requires education of fruit growers to demonstrate that effective results are obtained by persistence and commitment.

The following comment reflects how important it is to consider who our neighbours are, what attitudes are influencing them in the adoption of fruit fly control and how capable they see themselves in participating in the fight to control medfly:

“We also have the issue that our neighbour does nothing, so no matter how hard we try, we will always have an issue beyond our boundary fence” (comment from MedflyMotivations survey, 2014).

## **Acknowledgements**

The authors would like to acknowledge the support of the Australian Government’s Cooperative Research Centres Programme.

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### Annex. Scoring key for data analysis

	Question	Range answers	Items requiring reverse scoring	Items requiring internal consistency analysis	Items requiring multiplication	Constructs
<b>9a</b>	I intend to control fruit fly on my property this year	1 - 5				<b>Intention</b>
<b>14e</b>	I believe that controlling fruit fly on my property protect other people's fruit trees	1 - 5				<b>Attitude (Behavioural Belief)</b>
<b>11a</b>	demanding vs easy	(-2,2) from 1 to 7		<b>11a</b>		<b>Attitude</b>
<b>14i</b>	Controlling fruit fly requires a lot of time and help	1 - 5			14i x 18e	<b>Behavioural Beliefs</b>
<b>18e</b>	Having extra help and time to apply control treatments	-2, +2				<b>Outcome evaluation</b>
<b>11b</b>	complicated vs simple	(-2,2) from 1 to 7		<b>11b</b>		<b>Attitude</b>
<b>15d</b>	If I use a single type of control treatment, I will be able to control fruit fly	1 - 5			15d x 18f	<b>Behavioural Beliefs</b>
<b>18f</b>	Applying several methods simultaneously	-2, +2				<b>Outcome evaluation</b>
<b>11c</b>	worthless vs rewarding	(-2,2) from 1 to 7		<b>11c</b>		<b>Attitude</b>
<b>14g</b>	My neighbour's fruit trees are not affected by what I do on my property	1 - 5			14g x 15f	<b>Behavioural Beliefs</b>

	Question	Range answers	Items requiring reverse scoring	Items requiring internal consistency analysis	Items requiring multiplication	Constructs
<b>15f</b>	My neighbour will have less fruit fly on their property if I control fruit fly on mine	-2, +2				<b>Outcome evaluation</b>
<b>15e</b>	Controlling fruit fly on my property will help eliminate fruit fly from my area/ region	1 - 5			15e x 18b	<b>Behavioural Beliefs</b>
<b>18b</b>	Eliminating fruit fly from my area/ region	-2, +2				<b>Outcome evaluation</b>
<b>14k</b>	Controlling fruit fly on my property has little impact to the fruit industry in Western Australia	1 - 5			14k x 17b	<b>Behavioural Beliefs</b>
<b>17b</b>	Controlling fruit fly on my property will reduce the problems to the fruit industry in Western Australia	-2, +2				<b>Outcome evaluation</b>
<b>11d</b>	effective vs ineffective	(-2,2) from 1 to 7	11d	<b>11d</b>		<b>Attitude</b>
<b>15c</b>	I will apply control treatments if they eliminate fruit fly immediately	1 - 5			15c x 18d	<b>Behavioural Beliefs</b>
<b>18d</b>	Eliminating fruit flies quickly	-2, +2				<b>Outcome evaluation</b>
<b>11e</b>	affordable vs expensive	(-2,2) from 1 to 7	11e	<b>11e</b>		<b>Attitude</b>

	Question	Range answers	Items requiring reverse scoring	Items requiring internal consistency analysis	Items requiring multiplication	Constructs
<b>15b</b>	If fruit fly control treatments are expensive I will not use them	1 - 5			15b x 18c	<b>Behavioural Beliefs</b>
<b>18c</b>	Using expensive control methods	-2, +2				<b>Outcome evaluation</b>
<b>11f</b>	harmful to health vs safe to health	(-2,2) from 1 to 7		<b>11f</b>		<b>Attitude</b>
<b>15a</b>	If I spray chemicals, I will be able to control fruit fly	1 - 5			15a x 18a	<b>Behavioural Beliefs</b>
<b>18a</b>	Producing fruit without chemical residues	-2, +2				<b>Outcome evaluation</b>
<b>14a</b>	People who are important to me think that I should apply fruit fly control treatments on my property	1 - 5		<b>14a</b>		<b>Subjective Norm</b>
<b>19a</b>	Most fruit growers believe everybody should control fruit fly on their property	-2, +2			19a x 16a	<b>Normative Belief</b>
<b>16a</b>	(I will control fruit fly on my property of asked by a...) fruit grower	1 - 5				<b>Motivation to comply</b>
<b>14d</b>	At least one of my neighbours believes everybody should control fruit fly on their property	-2, +2			14d x 16b	<b>Normative Belief</b>

	Question	Range answers	Items requiring reverse scoring	Items requiring internal consistency analysis	Items requiring multiplication	Constructs
<b>16b</b>	(I will control fruit fly on my property of asked by a...) neighbour	1 - 5				<b>Motivation to comply</b>
<b>14b</b>	I am confident that I can control fruit fly if I wanted to	1 - 5		<b>14b</b>		<b>Perceived Control Behaviour</b>
<b>14c</b>	I am not confident with the use of baits to control fruit fly	-2, +2	14c		14c x 19e	<b>Control Belief Power</b>
<b>19e</b>	People are less likely to control fruit fly if baiting attracts more fruit flies onto their property	1 - 5				<b>Control Belief Strength</b>
<b>14f</b>	I am less likely to control fruit fly if treatments take too long to provide results	-2, +2			14f x 14h	<b>Control Belief Power</b>
<b>14h</b>	Spraying chemicals offer a quick solution to eliminate fruit fly on properties	1 - 5				<b>Control Belief Strength</b>
<b>14j</b>	I am less likely to implement control treatments if these are expensive	-2, +2			14j x 19d	<b>Control Belief Power</b>
<b>19d</b>	The cost of control treatments has an impact on how fruit fly control is done on properties	1 - 5				<b>Control Belief Strength</b>

	Question	Range answers	Items requiring reverse scoring	Items requiring internal consistency analysis	Items requiring multiplication	Constructs
<b>19b</b>	I am not confident with the use of traditional pesticides because of possible effects on health	-2, +2	19b		19b x 17a; 19b x 19f	<b>Control Power</b> <b>Belief</b>
<b>17a</b>	Organic chemicals are a only safe choice to control fruit fly on properties	1 - 5				<b>Control Strength</b> <b>Belief</b>
<b>19f</b>	Traditional pesticides are a safe choice to control fruit fly on properties	1 - 5				<b>Control Strength</b> <b>Belief</b>
<b>19c</b>	It is possible to get good results with control treatments that are easy to use	-2, +2			19c x 17c	<b>Control Power</b> <b>Belief</b>
<b>17c</b>	Cleaning properties is a very demanding option to control fruit fly	1 - 5				<b>Control Strength</b> <b>Belief</b>
<b>12a</b>	speed of results	1 - 5				<b>Control (direct)</b> <b>Belief</b>
<b>13a</b>	slow	1 - 5	-2, +2			<b>Control Power (direct)</b> <b>Belief</b>
<b>12b</b>	complexity	1 - 5				<b>Control (direct)</b> <b>Belief</b>
<b>13b</b>	complicated	1 - 5	-2, +2			<b>Control Power (direct)</b> <b>Belief</b>
<b>12c</b>	effectiveness	1 - 5				<b>Control (direct)</b> <b>Belief</b>
<b>13c</b>	irregular results	1 - 5	-2, +2			<b>Control Power (direct)</b> <b>Belief</b>



	Question	Range answers	Items requiring reverse scoring	Items requiring internal consistency analysis	Items requiring multiplication	Constructs
<b>12d</b>	cost	1 - 5				<b>Control (direct)</b> <b>Belief</b>
<b>13d</b>	expensive	1 - 5	-2, +2			<b>Control Power (direct)</b> <b>Belief</b>
<b>12e</b>	health safety	1 - 5				<b>Control (direct)</b> <b>Belief</b>
<b>13e</b>	toxic	1 - 5	-2, +2			<b>Control Power (direct)</b> <b>Belief</b>



# **Control Methods & Supporting Technology**

## **Integrating bait stations as an IPM component in area-wide fruit fly operational programmes**

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### **Abstract**

The concept of bait stations (BS) for control of economically-important fruit flies has received substantial attention in recent years. BS are being used as important components of area-wide IPM action programs that utilize the Sterile Insect Technique (SIT). BS are referred to as lure and kill, attract and kill, male annihilation, bait sprays, and attracticide/attracticidal. BS have been defined as “*discrete containers of attractants and toxins, which are targeted at specific pests*”. Desirable characteristics of BS include: 1) Ability to target and suppress female populations, 2) low cost in terms of attractant, killing agent and device itself, 3) attracted flies should not be trapped and retained, 4) long lasting attractant effects of the bait and long residual toxicity of the insecticide, resulting in reduced servicing or replacing and cost savings, 5) ease of use, disposable and/or biodegradable, 6) high selectivity i.e., no negative non-target effects and 7) positive benefit-cost relationship. BS can be utilized in infested areas for population suppression in specific sites where localized fruit fly reservoirs are present and in pest free or low prevalence areas for eradication of outbreaks as a component of an IPM approach. The paper presents the use of BS in area-wide fruit fly operational programs and presents a description of commonly used BS. It briefly discusses cost implications when BS are used as a stand-alone control tool and as part of an IPM approach. BS technology can be expensive, thus, its use in an area-wide IPM program is quite specific and requires careful technical and economic analysis. The paper provides a list of recommendations to improve BS use.

*Keywords:* bait stations, costs, fruit flies, IPM tools.

### **Introduction**

The concept of bait stations (BS) for control of economically-important fruit flies has received substantial attention in recent years. BS have been used for many years as important components of area-wide IPM action programs that utilize the Sterile Insect Technique (SIT). BS are referred to as lure and kill, attract and kill, male annihilation, bait sprays, and attracticide/attracticidal. For the purpose of this paper, the term “bait station” will be used throughout the text since it is the most widely used by action program managers. BS have been defined as “*discrete containers of attractants and toxins, which are targeted at specific pests*” Mangan and Moreno (2007). Desirable characteristics of BS include: 1) Ability to target and

suppress female populations; 2) low cost in terms of attractant, killing agent and device itself; 3) attracted flies should not be trapped and retained; 4) long lasting attractant effects of the bait and long residual toxicity of the insecticide, resulting in reduced servicing or replacement and less hand labor costs; 5) ease of use, disposable and/or biodegradable; 6) high selectivity i.e., no negative non-target effects; and 7) positive benefit-cost relationship. An additional characteristic of a BS is the inclusion of visual cues that are known to synergistically enhance the response of fruit flies to odor sources (IAEA, 2009).

### *Historical Perspective*

The use of BS as part of area-wide fruit fly IPM programs that integrate the SIT, has evolved considerably since its initial application in the early 1960's. The first successful BS application as part of an action program against a fruit fly pest was the combination of the powerful male-specific lure methyl eugenol (ME), with an organophosphate insecticide, which formed the basis of the male annihilation technique (MAT) against Oriental fruit fly (*Bactrocera dorsalis*) entries into mainland USA (Christensen, 1963). This BS has had the big advantage of using a powerful male specific parapheromone attractant, however, only useful for the ME responding *Bactrocera* species. For most other fruit fly species of economic and quarantine importance, BS have been based on protein based food attractants and visual clues or a combination of both. BS have been improved as a response to the need for lower cost, more efficient and selective BS systems for use in area-wide action programs. The first generation of protein based BS used in action programs appeared in the late 1970's (Programa Moscamed, 1984). These BS were short-lived, used liquid formulation of generic hydrolysate proteins which attract a wide-range of tefritid fruit flies and non-target organisms and killing agents based on broad spectrum organophosphate insecticides. Examples of first generation BS used to suppress Mediterranean fruit fly (*Ceratitidis capitata*, Wied.) and other fruit flies such as economic species of the *Anastrepha* genus (*A. ludens* and *A. obliqua*) include (Moscamed Programme, 1984): 1) a piece of corn cob impregnated with a solution of protein bait lure and malathion at a 4:1 proportion, and 2) a so-called "killing bag", which is a small bag made of natural fiber and filled with an absorbent material, such as wood chips, and soaked in a protein bait lure and malathion at a 4:1 proportion. These BS evolved into the second generation of BS, that are in general longer lasting, use dry or liquid synthetic food lures which are much more selective as well as more selective insecticides (Epsky et al., 2012). Second generation of BS include additional improvements such as the design of devices that do not retain captured adult flies reducing hand labor costs as well as rain proof and in certain cases biodegradable BS devices. Examples of these BS used widely in action programs targeting *C. capitata* include: 1) A bait station consisting of a 600 ml plastic bottle with side openings and baited with a sponge impregnated with 250 ml of a mixture of GF-120 (a.i. Spinosad) and water at a 1:4 proportion with a life-span of 4 weeks, 2) "Wax-BS" bait station consisting in a wax coated cardboard that contains a matrix composed of paraffin wax with yellow: green coloring added to provide a visual cue, corn syrup and granulated sugar as feeding stimulants, and an organic

toxicant (Tracer 120 SC (Spinosad)) coated on the wax. The BS is baited with ammonium acetate and trimethylamine lures (Biolure) and has a life-span of 6 to 8 weeks (Heath et al., 2013) and 3) the MagnetMed consisting of a paper enveloped attract-and-kill device impregnated with deltamethrin that contains two membrane dispensers (trimethylamine and ammonium acetate) as attractants with a life-span of up to 26 weeks (Navarro-Llopis et al., 2013). Other such BS include the insecticide-free bait station MS2 used in combination with the food based Cera Trap attractant in action programs in Mexico for suppression of *A. ludens* (de los Santos et al., 2011). These improvements have substantially increased cost-effectiveness of BS, thus, improving its range of applications and its feasibility for use in area-wide IPM action programs. A more comprehensive historical review of available BS and BS under development can be found in Piñero et al. (2014).

#### *Procedures for BS application*

In general, the use of BS as a stand-alone control tool is considered to be inappropriate. This is because of the large number of BS that would need to be used in order to suppress populations to an acceptable level, greatly increasing hand labor and material costs and affecting the economic feasibility of program implementation and of fruit production and commercialization.

Effective use of BS requires integrating this tool to other control tools in an area-wide IPM approach. BS should be applied on an area-wide basis and used for population suppression to complement other population suppression tools such as bait sprays, fruit stripping, orchard sanitation, biological control with parasitoids, and the sterile insect technique (SIT). When used as part of an IPM approach that integrates SIT, BS as well as other complementary population suppression methods should be used prior to sterile insect releases to prevent the killing of the sterile insects. Sterile insects are aimed at eradicating the pest population or maintaining populations at low prevalence levels, after the populations have been reduced to low levels through the use of suppression methods such as the BS.

Therefore, BS should be applied in the following situations (IAEA, 2009):

1. In infested areas for population suppression in localized pest reservoirs that occur in commercial fruit orchards and marginal host areas. Infested areas may include natural parks with presence of fruit hosts as well as organic fruit production.
2. In infested areas for population suppression in rural and urban sites. Infested areas may include backyard hosts and sites where large volumes of fruits are gathered such as fruit markets.
3. In pest free areas as part of a contingency plan for eradication of pest outbreaks.
4. In pest free areas, preventatively, where pest entries are recurrent. This application requires knowledge of population ecology for appropriate timing in the deployment of BS in the field. To optimize its application, BS need to be out in the field prior to the presence of the pest in the target area.

5. As a general rule, BS should not be applied for population suppression in large extensions of commercial fruit orchards or in marginal areas with continuous hosts over large extensions of land.

BS can be used in an IPM approach with competitive advantages over ground and aerial bait sprays in the following situations (Programa Moscamed, 2014):

1. In localized pest reservoirs during the rainy season. BS are in general rain proof, thus, remain effective in the field during heavy rains compared to the bait sprays which are normally washed away requiring additional treatments.
2. In backyard fruit hosts since BS is a less intrusive method that can remain active for at least eight weeks requiring one or two treatments, compared to weekly treatments in the case of ground bait sprays.
3. In organic fruit production since baits and killing agents are not sprayed into the environment but are contained in the BS body.
4. In natural protected areas for the same reason as above.
5. In difficult to access sites as only one or two treatments would be required.

### **Technical criteria for BS application**

BS applications described in this document are based on practical experience of large-scale fruit fly control programs.

#### *In pest reservoirs within infested areas*

BS should be applied during three biological cycles of the pest in the total infested area (usually no more than 1 km<sup>2</sup>). In the case of commercial orchards or marginal areas with continuous fruit hosts, a density of 50 BS/ha should be used. In areas with scattered fruit hosts, a density of 15 to 25 BS/ha should be used. For long-lasting BS, 2 treatments in order to cover three cycles of the pest is sufficient as BS remain active for 6 to 26 weeks. For short-lasting BS, 6 to 12 treatments will be required since replacement will be necessary once every week or every two weeks. BS deployment will greatly depend on the spatial distribution of fruit hosts. For commercial fruit orchards or marginal areas with continuous hosts, a uniform BS array should be used with BS placed equidistant. For areas with scattered fruit hosts, BS are placed in irregular arrays following the distribution of the hosts.

#### *In isolated or multiple outbreaks in pest free areas (PFA) or areas of low pest prevalence (LPA)*

BS should be applied during two biological cycles of the pest in the first square kilometer (100 ha) of the outbreak. In PFA and LPA with scattered fruit hosts, a density of 15 to 25 BS/ha should be used. In PFA with continuous hosts, 25 hectares should be covered and 20 hectares in LPA using the same range of BS density. For long-lasting BS, 1 treatment in order to cover two cycles of the pest is sufficient. For short-lasting BS, 4 to 8

treatments will be required since replacement will be necessary once or twice per week. BS deployment follows the same criteria as in the previous case.

The number of bait stations per hectare as well as the number of treatments required may be optimized with reliable information on fruit fly spatial and temporal distribution within and outside a commercial fruit orchard.

### **Projecting BS costs**

In utilizing BS in area-wide fruit fly control programs costs become an essential factor. In order to provide a general view of the costs of the utilization of this control method, a cost analysis was conducted comparing the use BS as a stand-alone control technique with BS used as part of a fruit fly IPM program (Table 1). An average cost of US \$3 per BS was established based on current costs of BS commercially available. The unit cost includes attractant, BS container with accessories, hand labor and fuel. The cost of the attractant, BS container and accessories are sold by distributors worldwide and are pretty much standard, whereas, the hand labor and fuel will depend on specific countries or regions. In this case, the hand labor and fuel costs are relatively low and correspond to the wage of a field technician and a liter of fuel in Central America.

From projecting BS costs it becomes clear that using BS as a stand-alone control tool for population suppression in reservoirs or to eradicate populations in an outbreak is much more expensive and probably less effective than using BS as part of an IPM approach. The use of BS alone in the best case is 2.73 fold more expensive and in the worst case 6.72 fold, compared to its use as part of an IPM approach (Table 1).

### **Recent developments**

Efforts to develop more effective long lasting and cheaper BS to be used as part of area-wide fruit fly IPM programs continue (Ekesi et al., 2007; IAEA, 2007; Piñero et al., 2009; Navarro-Llopis et al., 2015). One such BS is the autodissemination BS developed against *C. capitata*. Two BS models have been evaluated: The cylindrical type is composed of a 500 ml plastic (polyethylene terephthalate) bottle (14.0 cm high x 8.5 cm diameter) with fifteen 2.5 mm holes evenly distributed on the sides, a lid containing four triangular openings of 1.5 mm on each side, and an open bottom. The basket with the TML plug is placed inside hanging from the top. The lid and the bottom are covered with tulle fabric, and the outside of the device is fully covered with a yellow plush fabric (14 cm x 22 cm) impregnated with 2 g of *B. bassiana* conidia. The rectangular panel composed of a galvanized panel (23 cm x 14 cm) with a basket and a TML plug inserted in a 2.5 hole in the center of the panel. This device is also covered with yellow plush fabric (23 cm x 14 cm) impregnated with 2 g of *B. bassiana* conidia. According to Flores et al. (2013), to effectively disseminate the *B. bassiana* conidia to *C.*

**Table 1.** Cost comparison between the use of bait stations as a stand-alone control tool and as a part of an area-wide IPM approach.

		No.	BS						Total Cost/km²/yr (USD)				
	No. BS		Treat.	Unit	BS	Ground	SIT	Other	Surveillance	1	10	100	Cost
Application	Scenario	km²	Cost (USD)		Cost	Spray	km²/yr	Control	km²/yr	(100 ha)	(1000 ha)	(10,000 ha)	Ratio
					km²/yr	km²/yr		Costs					
					km²/yr	km²/yr		km²/yr					
Reservoirs:													
Continues hosts	Stand alone	10000 <sup>1</sup>	6 <sup>9</sup>	3	180,000	0	0	0	104 <sup>22</sup>	180,104	1,801,040	18,010,400	4.65
	IPM	5000 <sup>2</sup>	2 <sup>10</sup>	3	30,000	4,000 <sup>13</sup>	3,640 <sup>17</sup>	1,000 <sup>21</sup>	104	38,744	380,744	3,874,400	
Scattered hosts	Stand alone	5000 <sup>3</sup>	6	3	90,000	0	0	0	104	90,104	900,104	9,001,040	6.72
	IPM	1500 <sup>4</sup>	2	3	9,000	2,000 <sup>14</sup>	1,820 <sup>18</sup>	500	104	13,424	134,240	1,342,400	
Outbreaks:													
Continues hosts	Stand alone	2500 <sup>5</sup>	2 <sup>11</sup>	3	15,000	0	0	0	240 <sup>23</sup>	15,240	152,400	1,524,000	3.01
	IPM	625 <sup>6</sup>	1 <sup>12</sup>	3	1,875	2,000 <sup>15</sup>	840 <sup>19</sup>	100	240	5,055	50,550	505,500	
Scattered hosts	Stand alone	1250 <sup>7</sup>	2	3	7,500	0	0	0	240	7,740	77,400	774,000	2.73
	IPM	375 <sup>8</sup>	1	3	1,125	1,000 <sup>16</sup>	420 <sup>20</sup>	50	240	2,835	28,350	283,500	

<sup>1</sup>In the case of pest reservoirs, when used as a stand-alone tool, the average BS density in an area with continuous hosts (commercial orchard or marginal area) is 100 BS/ha.

<sup>2</sup>In the case of pest reservoirs, when used as part of an IPM approach in a continuous host situation, the recommended density is 50 BS/ha.

<sup>3</sup>In the case of pest reservoirs, when used as a stand-alone tool, the average BS density in an area with scattered hosts is 50 BS/ha.

<sup>4</sup>In the case of pest reservoirs, when used as part of an IPM approach, the average BS density in an area with scattered hosts is 15 BS/ha.

<sup>5</sup>In the case of outbreaks and when BS are used as a stand-alone control tool in a continuous host situation, the recommended density is 100 BS/ha in 25 ha around the outbreak.

<sup>6</sup>In the case of outbreaks and when BS are used as part of an IPM approach in a continuous host situation, the recommended density is 25 BS/ha in 25 ha around the outbreak.

<sup>7</sup>In the case of outbreaks and when BS are used as a stand-alone control tool in an area with scattered fruit hosts, the recommended density is 50 BS/ha in 25 ha around the outbreak.



<sup>8</sup>In the case of outbreaks and when BS are used as a part of an IPM approach in an area with scattered fruit hosts, the recommended density is 15 BS/ha in 25 ha around the outbreak.

<sup>9</sup>Each treatment is effective for 8 weeks. Since the application is in a pest reservoir and BS are used as a stand-alone method, to cover the 52 weeks of the year at least 6 treatments are required.

<sup>10</sup>Same as above, however, because BS is applied as part of an IPM approach only 12 weeks need to be covered with BS, thus, 2 treatments are sufficient.

<sup>11</sup>Each treatment is effective for 8 weeks. Since the application is in a pest outbreak and BS are used as a stand-alone method, to cover 3 cycles of the pest (12 weeks), 2 treatments are required.

<sup>12</sup>Same as above, however, because BS is applied as part of an IPM approach, to cover 2 cycles of the pest (8 weeks), only 1 BS treatment is required.

<sup>13</sup>To cover 2 cycles in continuous host situation, 8 weekly treatments x US \$5/treatment x 100 ha (1 km<sup>2</sup>).

<sup>14</sup>Half the amount of product required in scattered fruit hosts thus half the cost.

<sup>15</sup>In an outbreak situation one cycle is to be covered, thus, 4 weekly treatments are required.

<sup>16</sup>Half the amount of product required in scattered fruit hosts thus half the cost.

<sup>17</sup>US \$3,640 (2000 SF/ha x 100 ha = 200,000 SF/km<sup>2</sup>; US \$350 per million produced and released SF (US \$70 per 200,000 SF in 1 km<sup>2</sup>)). US \$70 x 52 weeks to cover one year release = US \$3,640.

<sup>18</sup>50% the density used in a scattered host situation.

<sup>19</sup>US \$840 (US \$70/km<sup>2</sup> x 12 weeks). 12 weeks required to cover 3 cycles of the pest.

<sup>20</sup>50% the density used in a scattered host situation.

<sup>21</sup>Includes low cost methods such as orchard sanitation and fruit stripping.

<sup>22</sup>US \$104 (2 traps/km<sup>2</sup> x US \$2/trap x 26 weeks service).

<sup>23</sup>US \$240 (10 traps/km<sup>2</sup> x US \$2/trap x 12 weeks service (3 cycles)).

*capitata* wild population, one bait station per hectare must be installed, and the conidia-treated fabric must be replaced every 15 days. These bait station devices have been evaluated in open field tests against *C. capitata* in Guatemala. The results are promising and show a significant population suppression effect when integrating the autodissemination BS to an IPM approach including the SIT.

The dissemination of the fungus conidia is very specific as only *C. capitata* males responding to trimedlure will approach the *B. bassiana* inoculated bait station and become infected. This technology has a multiplicative effect, since inoculated wild males will infect other males and females during courtship. Because of its mode of action, this technology is considered to be species specific and environmentally friendly (Flores et al., 2013).

## Conclusions

Bait stations can be used as a component of an area-wide IPM approach in infested areas for population suppression in specific localized fruit fly reservoirs and in pest free and low prevalence areas for eradication of outbreaks.

The use of BS as a stand-alone control tool is much more expensive and less effective than when used in combination with other control tools in an IPM approach.

BS technology can be expensive, thus, its use in an area-wide IPM program is quite specific and requires careful technical and economic analysis.

The greatest potential of bait station technology is when population densities are still at low levels. Thus, it is essential to assess fruit fly population fluctuation and spatial distribution in order to deploy BS at the right time and place.

The efficacy of BS compared to other control methods will greatly depend on the effectiveness to attract and kill and its lifespan in the field. Ideally, effectiveness of BS should last throughout the fruiting season of the crops.

## Recommendations

Develop new, more powerful and long-lasting attractants that can increase bait station effectiveness.

Develop effective and environmentally-friendly killing agents (e.g. entomopathogens), and integrate visual and olfactory cues.

Detailed knowledge of fruit fly population ecology is essential for timing the deployment of BS in the field as well as for assessing the spatial distribution of BS. If fruit fly spatial distribution within a commercial orchard or in marginal host areas is known, bait stations may be aggregated to overlap with the fruit fly population. Knowledge of the dispersion behavior of fruit flies from areas surrounding the orchard into the orchard, may be used to deploy bait

stations around the orchard's periphery before the flies move into the orchard to reduce or eliminate immigrating flies (Alemany et al., 2004).

Economic feasibility assessments of the use of BS is required to support decision making between the use of this technology and other alternate technologies aimed at fruit fly population suppression. Non-target effects to demonstrate the environmental benefits of BS should be part of the variables to quantify in the assessment.

Conducting side-by-side comparisons of the various bait station types that have been developed in recent years to determine actual effectiveness against multiple fruit fly species in various geographical areas and using standardized methodologies. BS evaluation must ultimately be based on fruit infestation levels.

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## **Integrated Pest Management for *Bactrocera dorsalis* (Hendel) and *Bactrocera zonata* (Saunders) on Kinnow Mandarin in the Indian Punjab**

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### **Abstract**

*Introduction:* Kinnow mandarin, a hybrid between King (*Citrus nobilis*) and Willow leaf (*Citrus deliciosa*) mandarins is the prime citrus fruit of Indian Punjab. Every year during August and September, *Bactrocera dorsalis* and *Bactrocera zonata* cause damage to 40-80 % of harvested fruits of Kinnow mandarin. Both fruit fly species are difficult to manage as they are polyphagous, multivoltine, with highly mobile and fecund adults with immature stages that are protected inside fruits. Since applications of insecticides disrupt the agroecosystem, we investigated the potential to control these flies with an integrated pest management (IPM) approach.

*Materials and Methods:* Eco-friendly fruit fly management techniques comprising of ploughing, sanitation, male annihilation technique (using methyl eugenol based traps) and poison bait application technique (use of protein hydrolysate bait with insecticide spinosad) were used during 2010-2012 at the University Seed Farm Kinnow mandarin orchards. These integrated management treatments were applied in a systematic manner in one acre of Kinnow mandarin orchard and each treatment was replicated three times in three different acres in the same location. In another one acre orchard, the existing recommendations (by Punjab Agricultural University (PAU), Ludhiana) for fruit fly management were applied and an orchard of same area served as untreated control.

*Results:* The results revealed that the IPM treatments significantly reduced fruit fly infestation on Kinnow mandarin crop. Fruit infestation in the IPM treated orchard, existing recommendations and the control was 4.6 per cent, 34.5 per cent and 50.5 per cent, respectively. The number of larvae per fruit was significantly lower in the IPM treated orchards (9.2/fruit) compared to the existing recommendations (17.1/fruit) and the control orchard (26.2/fruit). The males captured per trap in the IPM orchard varied from 161.00 early in the season to 3364.30 later at the onset of winter. The fruit yield was significantly higher in the IPM orchard compared to the two other orchards. Likewise, the net income/acre was higher in the IPM orchard. The cost-benefit analysis showed about two times benefit in adopting IPM over following the existing recommendations by PAU. The results clearly revealed that IPM significantly reduced the infestation of fruit flies in Kinnow mandarin. This means that IPM could help Kinnow growers of Punjab to increase the yield of quality marketable fruits by reducing the fruit fly infestation along with reduced dependence on insecticides.

*Keywords:* integrated pest management, fruit flies, Kinnow mandarin.

## Introduction

Kinnow mandarin, a hybrid between King (*Citrus nobilis*) and Willow leaf (*Citrus deliciosa*) mandarins is an important fruit crop of Punjab occupying 45850 ha with 988000 metric ton production (PAU, 2014). During August and September, two important fruit flies, *Bactrocera dorsalis* and *Bactrocera zonata* destroy 40-80 % fruits of Kinnow mandarin (Singh & Sharma, 2013). These important pests are difficult to manage as they are polyphagous, multivoltine, with highly mobile and fecund adults with immature stages that are protected inside fruits (Sharma et al., 2011; Singh & Sharma, 2013).

Application of insecticides disrupts the ecosystem and causes numerous hazards, which in the present scenario warrants the need of integrated approach for fruit fly management (Verghese et al., 2012). The use of methyl eugenol traps stands as the most outstanding alternative among the various techniques available for the effective management of fruit flies when combined with other IPM practices. Methyl eugenol, when used together with an insecticide impregnated into a suitable substrate, forms the basis of male annihilation technique (MAT) (Manrakhan et al., 2014). MAT using methyl eugenol traps @ 4 traps/ acre in mango and guava orchards has been found to be more effective in managing fruit flies in different parts of India along with bait application technique (BAT) (Stonehouse et al., 2007). Singh and Sharma (2011) compared the trapping efficiency of differently shaped methyl eugenol based traps in Kinnow mandarin in Punjab and found that methyl eugenol soaked plywood blocks when used in transparent mineral water bottle traps (103.2 flies/trap) were more efficient than when used in McPhail trap (63.8 flies) and Nomate trap (59.5 flies) in capturing the male fruit flies.

Female fruit flies feed on protein to mature sexually and for the development of their eggs (McPhail, 1939; Christenson & Foote, 1960). Hence, female attractive baits are used to control the females (bait application technique, BAT) (Steiner, 1952; Mazor et al., 2002). Female targeted system normally consists of sprays/traps baited with a liquid solution made from protein and fermenting sugar (Epsky et al., 1999; Ferrar, 2010). Rajita and Viraktamath (2005) evaluated different protein sources attracting the female fruit flies in guava and mango, and found that yeast and soybean were very attractive to *B. dorsalis*. The use of spinosad based baits for attracting and killing of female fruit flies on different fruit crops is gaining importance (King & Hennessey, 1996; Raga et al., 2003; Ho et al., 2008; Vayssieres et al., 2009; Yee, 2011) as this technology has been found environmentally safe (Vargas et al., 2001, 2008; William et al., 2003; Mangan & Moreno, 2007). Protein baits attract both male and female fruit flies, making them more effective than the MAT (Sabine, 1992). GF-120 NF Naturalyte fruit fly bait has been approved for organic fruit and vegetable production against fruit flies in USA (Dow AgroSciences, 2009).

There have been several studies on the different fruit fly management techniques (Stonehouse et al., 2007; Vargas et al., 2010; PAU, 2014) which include cultural practices, MAT, bait application technique and chemical control. Application of the techniques separately did not give good results. Since application of insecticides disrupt the agroecosystem, we investigated

the potential to control these flies with an integrated pest management approach, comprising of ploughing, sanitation, MAT @ 16 traps/acre and BAT (plant based protein hydrolysate 0.1% + spinosad 0.03%) and existing fruit fly management recommendations comprising of ploughing, sanitation and fenvalerate sprays together with untreated control during 2010-2012 seasons.

## Materials and Methods

### *Preparation of fruit fly trap*

One litre capacity transparent mineral water bottles, converted into methyl eugenol based fruit fly traps were used in this experiment. The trap consisted of a plywood dispenser (MAT block), suspended vertically inside the bottle, aligning with the four vents that allow entry of fruit flies inside the bottle. The MAT block was prepared by soaking plywood blocks (7.5 cm x 6.0 cm x 2.0 cm) in a solution of ethyl alcohol, methyl eugenol (98%, Sisco Research Lab., Mumbai) and malathion 50 EC mixed in the ratio of 6:4:1 (v/v) for 72 hrs.. A hole in the MAT block was made with the help of a drill to put a wire through it, which was used to hang it inside the bottle, with loose ends of wire passing through the lid of the bottle and used to tie the trap on tree. Four holes were made with the help of a hot iron rod on the upper side of the bottle (near neck) for entry of fruit flies. Bottles were cut from bottom side with knife and MAT block was hanged inside the bottle with two sides of wire coming out from the top of the bottle. Four random holes of 3-4 mm diameter were made at the bottom with hot needles to drain the water that may get collected in the bottles. The wire was twisted to make a loop. The baited bottles were hanged on the trees at a height of 1-1.5 above ground level. Care was taken while hanging the traps, so that these were not directly exposed to the sunlight. Red coloured reflecting tape was tied to the trees on which traps were fixed for easy accessibility of such trees in the orchards. These traps were kept in the orchards till the fruit harvesting was over. Every week during servicing, the lower cut portion of the bottle was removed and all the fruit flies trapped in the bottle were placed in a carry bag. The lower cut portion of the bottle was again fixed with the bottle. The carry bags were labelled with a marker and fruit flies trapped/trap were counted in the laboratory. In cases where the number of fruit flies was large, the count was made on weight basis (for standardization, average dry weight of 100 adult flies, taken after keeping them for 3 days after collection at room conditions, was 450 mg or 4.5 mg/adult).

### *Treatments*

To develop a module of IPM treatments, the below mentioned treatments were superimposed in a systematic manner in one acre field and each treatment was replicated thrice. One acre field was kept as untreated control in which no such practice was done.

- T<sub>1</sub> - Ploughing with discs in June followed by regular removal of infested fallen fruits and burying same in 60 cm deep pit. The pit was covered after 2-3 days.
- T<sub>2</sub> - Methyl eugenol based 16 traps/acre fixed in last week of August
- T<sub>3</sub> - Protein hydrolysate (0.1%) + spinosad (0.03%) superimposed in 2<sup>nd</sup> week of September
- T<sub>4</sub> - Untreated control

The plot size was one acre. Each treatment was replicated three times.

### *Sampling and observations of fruit flies and infested fruits*

The number of fruit fly species trapped were collected and counted at weekly intervals. For obtaining fruit fly infestation percentage, from each treatment, a sample of 50 fruits from different trees at random was collected at weekly intervals. These fruits were sorted out as infested (based on pinhole size oviposition puncture) and healthy fruits. The per cent fruit infestation was worked out. Data were also recorded for number of larvae/fruit by dissecting the fruits.

### *Yield calculation*

Impact of different management strategies on the quality of marketable fruits was also assessed by taking yield data from 5 randomly selected trees in each treatment. Cost: benefit ratio of IPM treatments in relation to other management strategies was also worked out by taking the expenditure on ploughing, regular removal of fallen fruits and burying, fixing of traps (MAT), spraying of protein bait mixed with spinosad (BAT) and fenvalerate spray.

### *Statistical analysis*

Trap catches, per cent fruit infestation and number of larvae/fruit were subjected to completely randomized block design (CRBD) analysis, after suitable conversions of the data (arc sine, percentage and  $\sqrt{n}$  transformations), using statistical analysis software CPCS1.

## **Results**

### *Fruit infestation*

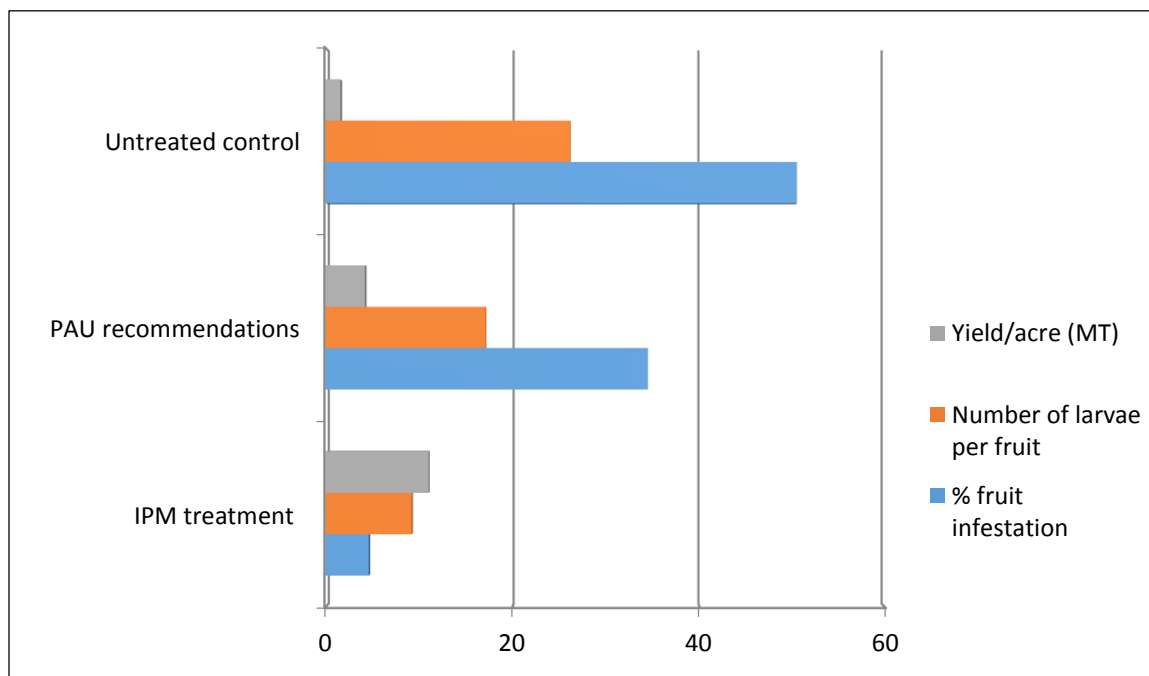
The data on per cent fruit infestation of Kinnow mandarin is represented according to the standard meteorological weeks (SMW) in Table 1. The mean values of three replications of each treatment represents that the fruit infestation was the lowest in IPM treatments (4.6 %) followed by existing recommendations of Punjab Agricultural University (PAU) (34.5 %) and untreated control (50.5 %) (Fig. 1). Hence, results demonstrate that the IPM treatments have a significant positive impact on reduction of fruit fly infestation of Kinnow fruits.



**Table 1.** Impact of different management strategies on *Bactrocera* spp. fruit infestation of Kinnow mandarin

Treatment	% infested fruits*														Pooled mean
	SMW**														
	32	33	34	35	36	37	38	39	40	41	42	43	44	45	
IPM	0.67	2.00	2.67	3.33	4.67	5.33	6.00	7.33	8.67	8.67	10.00	3.33	0.67	0.67	4.6
PAU	4.67	18.67	26.67	32.00	34.67	38.00	42.00	47.33	52.67	58.00	62.67	48.00	26.67	4.67	34.5
Untreated control	14.00	28.67	35.33	40.67	44.00	48.67	54.67	68.00	75.33	81.33	86.67	68.67	50.67	10.67	50.5
Critical difference (CD)# (p=0.05)	3.85	3.67	3.31	2.78	2.35	2.35	3.22	4.36	2.79	3.19	4.05	2.75	6.75	3.68	8.77

\*Mean of 3 replications; \*\*SMW-standard meteorological week; <sup>#</sup>Analysis was done with transformed (arc sine % transformation) data.

**Fig. 1.** Impact of different treatments on yield, larval population and fruit infestation of Kinnow mandarin.

#### Number of larvae/fruit

The mean number of larvae (Table 2) of *Bactrocera* species infesting Kinnow fruits revealed that in IPM treatments (9.2 larvae/fruit), the larval infestation was the lowest followed by existing recommendations (17.1 larvae/fruit) and untreated control (26.2 larvae/fruit) (Fig. 1). Thus, overall means indicated that larvae/fruit were significantly low in new IPM treatments as compared to existing recommendations and untreated control.

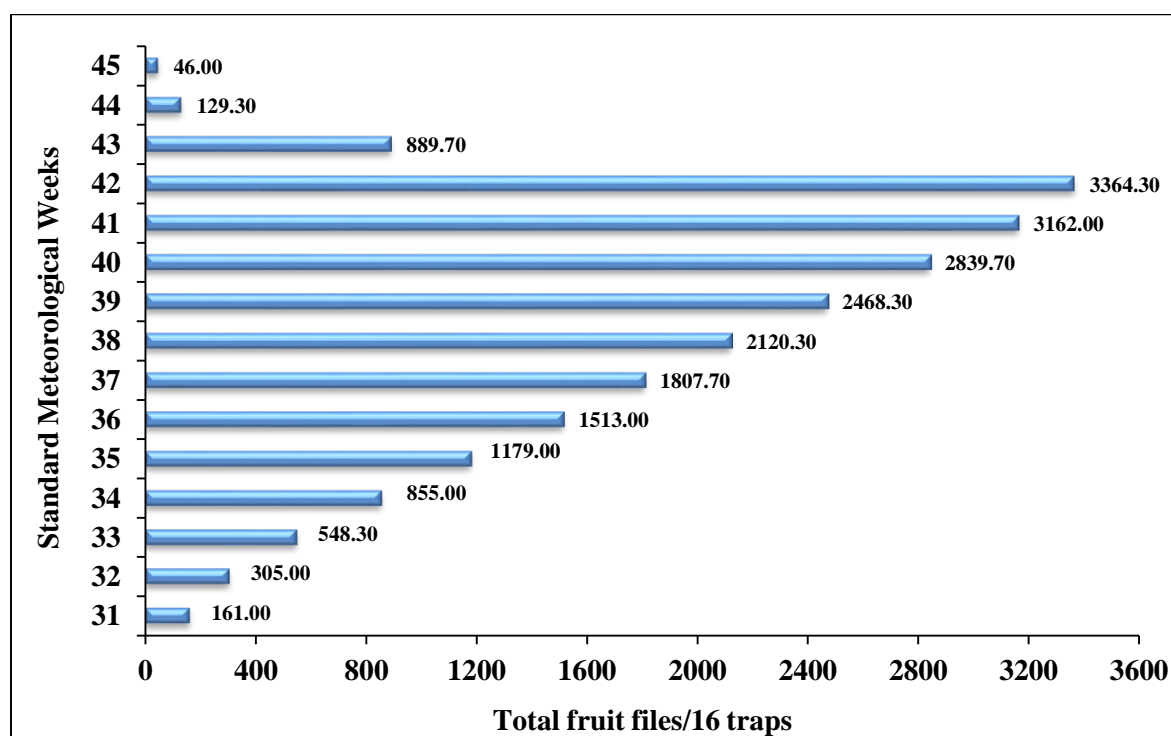
### Number of males captured

The males captured in 16 traps/acre in IPM treatments showed a progressive significant increase (Fig. 2). The males captured during different weeks varied from 161.00 (31<sup>st</sup> SMW) to 3364.30 (42<sup>nd</sup> SMW) but after 43<sup>rd</sup> SMW, the population declined and reached at 46.00/trap in 45<sup>th</sup> SMW.

**Table 2.** Impact of different management strategies on the number of larvae of *Bactrocera* spp. in Kinnow mandarin

Treatments	Number of larvae per fruit*									Pooled Mean
	SMW**									
	34	35	36	37	38	39	40	41	42	
IPM treatments	4.67	6.00	8.33	11.43	14.10	16.20	17.67	2.33	2.33	9.2
PAU recommendations	6.83	13.03	14.67	18.17	20.37	25.43	28.10	16.10	10.77	17.1
Untreated control	13.70	17.33	24.63	29.03	32.70	37.10	39.93	23.50	18.23	26.2
Critical difference (CD) <sup>#</sup> (p=0.05)	0.31	0.92	0.29	0.23	0.24	0.24	0.26	1.28	0.32	0.86

\*Mean of 3 replications; \*\*SMW-standard meteorological week; <sup>#</sup>Analysis was done with transformed ( $\sqrt{n}$  transformation) data.



**Fig. 2.** *Bactrocera* spp. males captured per 16 traps/acre from IPM treatments in Kinnow mandarin orchards.

### Number of marketable fruits, fruit yield and yield per acre

The impact of different management strategies adopted for reduction of *Bactrocera* species on Kinnow fruits indicated that in IPM treatments, there were 540.8 marketable fruits/tree in comparison to 206.0 in PAU recommendations and 76.4 in untreated control plots (Table 3). Similarly, the fruit yield (kg/tree) and yield/acre (MT) was significantly higher in case of IPM treatments (100.05 kg/tree and 11.01 MT) as compared to PAU recommendations (38.12 kg/tree and 4.20 MT) and untreated control (14.14 kg/tree and 1.56 MT) (Fig. 2).

**Table 3.** Impact of different management strategies for *Bactrocera* spp. on the quality of marketable fruits of Kinnow mandarin

Treatments	No. of marketable fruits/tree*	Fruit yield (kg/tree)*	Yield/acre (MT)
IPM treatments	540.80	100.05	11.01
PAU recommendations	206.00	38.12	4.20
Untreated control	76.40	14.14	1.56
CD <sup>#</sup> (p=0.05)	34.19	6.33	0.69

\*Mean of 5 trees; number of trees/acre=110; average weight of fruit=185 g; <sup>#</sup>CD, Critical difference.

### Cost: benefit ratio

The net income/acre was maximum in IPM treatments (Indian Rs. 1,26,386) in comparison to PAU recommendations (Indian Rs. 46,464) (Table 4). The cost: benefit ratio was quite high in IPM treatments (1:22) to that of 1:11 in PAU recommendations, which was almost 2 times more to that of PAU recommendations.

**Table 4.** Cost: benefit (C:B) ratio of IPM treatments in Kinnow mandarin in relation to other strategies

Treatments	Expenditure, Income and Cost: benefit ratio									
	Expenditure (Rs)						<sup>3</sup> Yield/acre (MT)	<sup>4</sup> Gross income (Rs/acre)	Net income (Rs.)	C:B ratio
	<sup>1</sup> Ploughing	<sup>2</sup> Regular removal of fallen fruits and burying	MAT (16 traps /acre)	BAT	Fenvalerate spray	Total				
	a	b	c	d	e	f	g	h	(h-f)	
IPM treatments	300	2000	996.75	2461	0	5757.75	11.01	1,32,144	1,26,386.25	1:21.9
PAU recommendations	300	2000	0	0	1987.50 <sup>†</sup>	4287.50	4.20	50,352	46,464.50	1:10.84
Untreated control	0	0	0	0	0	0	1.56	18,672	-	-
CD <sup>#</sup> (p=0.05)							0.69	8282.54	10790.9	

<sup>1</sup>Labour + tractor rent + diesel; <sup>2</sup>@ Rs. 1000/full season (6-7 pickings)/person; <sup>3</sup>number of trees/acre=110 and average weight of fruit=185 g; <sup>4</sup>@Rs.12/kg fruit; <sup>†</sup>5 sprays at weekly interval; <sup>#</sup>CD, Critical difference.

### Discussion and Conclusions

The most powerful technique for eradication/suppression of tephritids populations' is the simultaneous use of protein/malathion system and male annihilation. In western New South Wales, Australia, this method was observed to be more effective against *B. tryoni* than either technique applied alone (Bateman et al., 1966) and the population of fruit fly collapsed within five weeks of the commencement of the campaign on Easter Island (Bateman et al., 1973) which corroborate the present findings though the techniques applied were different but fruit fly infestation decreased significantly in both the situations. Similarly, in Nepal, fruit fly management programme which included farmers awareness, MAT and field sanitation received interest and support from the farmers for management of *B. dorsalis* (Pandey et al., 1997) whereas Yoshizawa (1997) described the successful eradication of fruit flies including *B. dorsalis* in Japan. Seewooruthun et al., (2000) used BAT and MAT for eradication of *B. dorsalis* in Mauritius.

Various workers (Hancock et al., 2000; Hurtrel et al., 2002; Allwood et al., 2003; Huang et al., 2006; Mau et al., 2007; Mwatawala et al., 2009; Vargas et al., 2010, Vayssieres et al., 2011; Srikachar et al., 2014) showed the successful control/reduction of different fruit fly species, viz. *B. dorsalis*, *B. zonata*, *B. papayae*, *B. correcta*, *B. cucurbitae*, *B. invadens*, *B. ciliatus*, *B. tryoni* and *C. capitata*. Singh (2004) also reported that combined management practices proved effective in reducing *B. dorsalis* populations in guava orchard in Punjab, whereas Verghese et al., (2004) assessed the effectiveness of a locally recommended IPM package in comparison with no control on a susceptible variety of mango.

Thus, the application of IPM treatments involving cultural practices, MAT and BAT in the present study successfully reduced the fruit fly population and infestation on Kinnow mandarin. This finding corroborates with the one obtained by Verghese et al., (2012) on mango in South India.

In conclusion, the results clearly revealed that IPM treatments significantly reduced the infestation of fruit flies in Kinnow mandarin. This means that IPM could help Kinnow growers of Punjab to increase the yield of quality marketable fruits by reducing the fruit fly infestation and dependence on insecticides. The use of IPM has proved to be beneficial for economical as well as environmental views, as it reduces un-judicial use of insecticides and hence increasing the cost-benefit ratio. It is safe to applicators and avoids environmental contamination with insecticide residues. These MAT traps and other IPM treatments used are affordable by the farmers and thus can be widely used for long-term management of fruit flies. Future research should continue to develop and test such techniques, which can be implemented in the IPM programmes.

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# Integrated Pest Management of fruit flies on Rose apple in Thailand

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## Abstract

**Background:** Rose apple is one of the most popular, nutritionally rich fruits with unique flavor, fragrance, taste, and health promoting qualities. It has great economic value and high export potential. However, rose apple is highly perishable fruit because of its thin skin and soft flesh. These two characteristics makes this fruit really susceptible to fruit fly attack. Rose apples might suffer significant damage up to 100 % if there are no appropriate control measures in place. The objective of this research is to increase the quality of rose apple using fruit flies integrated control measures.

**Methods:** Studies took place at the Plant Protection Research and Development Office laboratory and at rose apple orchards located at Nakhon Pathom and Ratchaburi provinces from September 2007 to October 2012. We divided the study into 3 steps: 1) fruit flies survey and determination of its seasonal abundance; 2) determination of ecological parameters of dominant; and 3) studying the fruit flies suppression technology.

**Results:** Three Tephritid specie were found infesting rose apple fruits, being *Bactrocera dorsalis* the dominat species. Seasonal abundance was determined with Steiner traps, showing two main population peaks, at fruit setting stage, and at harvest period. Rose apples appear to be highly susceptible about 21 days after stamens drop-off. Naturally infested Rose fruits surveillance also allowed the identification of two hymenopteran parasitoids, *Diachasmimorpha longicaudata* and *Forpius arisanus*. The biological and ecological study of the dominant species indicate that at  $23 \pm 1^\circ\text{C}$  *B. dorsalis* females became receptive to courting males approximately 8 days after emergence, being able of laying ~ 600 eggs during its life span, with nearly 87% average hatching. The larval stage lasted ~ 6 days, whereas pupa stage lasted ~ 9 days. Adult females and males were able to survive for  $95.0 \pm 11.9$  days, and  $97.5 \pm 9.3$  days respectively. Fruit fly IPM included orchard sanitation, chemical treatments with poison protein baits, mass-trapping, and physical barriers. Four different materials to be used as physical barriers were compared, the best results were obtained with polyester bags and white plastic bag of 40-45 microns thick.

**Conclusion:** Based on this research, we recommend the rose apples farmers to apply several measures to control fruit flies including (1) sanitizing orchards (2) using methyl eugenol for monitoring fruit fly population (3) using protein bait every 7 days for killing adult male and female, and (4) wrapping rose apple fruits with suitable bag 14 days after stamens fell off.

**Keywords:** integrated control, *Bactrocera dorsalis*, Rose apple.

## Introduction

Rose apple (*Eugenia juvaniea* Lamk) is one of the most popular, nutritionally rich fruits with unique flavor, fragrance, taste, and health promoting qualities (flowers are astringent and used to treat fever and halt diarrhea, but also show weak antibiotic action) in tropical Asia. It has great economic value and high export potential. However, the rose apple is highly perishable fruit because of its thin skin and soft flesh. By these characteristics, this delicate fruit is highly susceptible to Tephritid fruit fly infestation, which could also limit its trade potential. Indeed, Rose apples suffer significant damage, up to 100%, if there are no appropriate pest control measures in place.

Tephritid fruit flies are considered as major economic pests of various fruits, including rose apple, being most of them polyphagous species and present in tropical areas. Several works have reported that rose apples were attacked mainly by tephritid species belonging to *Bactrocera* genus: *Bactrocera dorsalis* (Hendel) (= syn. *Dacus dorsalis* Hendel), *B. correcta* (Bezzi), *B. carambolae* Drew and Hancock and *B. papayae* Drew and Hancock (Pholboon & Cantelo, 1975; Sen, 1986; Montree, 1999, 2001). Indeed, two of them have been considered of economic importance to two rose apples varieties, 'Toonklaow' and 'Sairong' (Sen, 1986).

To reduce this economic impact, the growers have to implement both pre- and post-harvest control measures, which also increase their production costs. Nowadays, chemical applications are being reduced due to the human health and environmental concerns (as pesticide residues in fruits or environment pollution). Whereas the phytosanitary (quarantine) restrictions imposed by Japan, USA, EU, Australia, New Zealand, Korea, Taiwan and China due to the presence of these fruit flies hinder the development of export markets. All together, these three main points, economic impact, environmental concern and quarantine measures, had pushed the Thailand government to get involved in the establishment and development of phytosanitary measures within an Integrated Pest Management program. To deal with this issue, it was advisable to start some basic research programs to increase the knowledge of fruit flies attacking rose apple and to determine best suited IPM measures for their control.

Linked directly with this last point, the main objective of this work was to study the biology, ecology and control method of economic important tephritid fruit flies on rose apple. The results will facilitate the exportation of rose apples in Thailand.

## Methods

Studies took place at the Plant Protection Research and Development Office laboratory and at rose apple orchards located at Nakhon Pathom and/or Ratchaburi provinces from September 2007 to October 2012.

*Survey of economically important fruit fly species on rose apples*

*Survey of fruit fly species on rose apples.* Samples of infested fruits from Rachaburi province and Nakhon Pathom province were collected and transferred to the laboratory of Pest Management Group, Plant Protection Research and Development Office, Department of Agriculture, Bangkok. The samples were weighted and counted. In addition, the information on date, stage of host plant and location was also recorded. All infested fruits were kept in plastic containers (22 x 29 x 10 cm) containing of saw dust 2.5 cm high for about 10 days, in a constant temperature and humidity room ( $23.10 \pm 1.27^{\circ}\text{C}$  and  $91.07 \pm 1.25\%$  RH). The maturing larvae left the fruits and pupated in saw dust. The pupae were collected by sieving saw dust through a 20-mesh screen sieve. Pupae were held in plastic containers (8 cm-diam. x 5 cm-high) covering with moist saw dust 1.5 cm high and subsequently placed in 16 mesh-wire screening cage (35 x 35 x 50 cm) until eclosion. Water and artificial diet for adult flies (consisting of a mixture of 4 parts of Brewer's yeast and 1 part of sugar) were placed in adult rearing cages. After 7-10 days from emergence, adult fruit flies were froze-killed in glass tubes for 4-5 hours. All emerged specimens were identified under stereo-microscope. Number of each fruit fly species and sex were recorded. Five surveys were carried out in each location.

*Determination of primary pest of rose apples.* The experiment was done in the same controlled conditions room ( $23.10 \pm 1.27^{\circ}\text{C}$  and  $91.07 \pm 1.25\%$  RH) using the methods of Southwood (1966). Fifty pairs of each fruit fly species of the same brood and age were kept in cages (19 x 30 x 20 cm). Five rose apples were placed in each cage for 1 hour, exposing to gravid females for oviposition. Subsequently, infested fruits were placed in plastic containers (22 x 29 x 10 cm) containing saw dust 2.50 cm high for 10 days until larvae entered pupation. A 20-mesh screen sieve was used to separate pupae from saw dust. Pupae were held in plastic containers (8 cm-diam. x 5 cm-high) covering with moist saw dust 1.5 cm high and subsequently placed in plastic boxes (22 x 29 x 10 cm). The following information was recorded: weight of test fruits, number of infested fruits, number and weight of pupae, number of adults and sex.

*Study on time for fruit fly infestation on rose apples.* The experiment was done on 2-year-old rose apple trees of 'Tabtimjan' cultivar at Amphur Samphan (Nakhon Pathom province). Ten fruits of the same age were randomly collected at 7, 14, 21, 28, 35 and 42 days after fruit set. Subsequently, all fruits were brought to the laboratory, weighted and measured for the size. Fruits were observed for signs of fruit fly infestation. If infested fruits were found, they were kept in plastic containers (22 x 29 x 10 cm) containing moist saw dust 2.50 cm high for 10 days until larvae entered pupation. A 20-mesh screen sieve was used to separate pupae from saw dust. Pupae were held in plastic containers (8 cm-diam. x 5 cm-high) covering with moist saw dust 1.5 cm high and subsequently placed in plastic boxes (22 x 29 x 10 cm). The following information was recorded: weight of fruits, number of infested fruits, number and weight of pupae, number and sex of adults (including identification of fruit fly species and other pest species or natural enemies).

*Study on seasonal abundance of fruit flies in the orchard.* The experiment was done on 1.5-year-old rose apple trees of 'Tabtimjan' cultivar at Amphur Duamnuan Sadork (Rachaburi province) and at Amphur Sampan (Nakhon Phatom province). Steiner traps with methyl eugenol lure mixed with malathion (Dimark 83 % EC) (at a ratio of 4:1) was used to attract fruit flies. Eight Steiner traps were placed covering an area of 1,600 square meters. Traps were hanged at 1.5 meters high on the trees. Traps were revised in a weekly manner, identifying each specimen to the closest species. Traps were established during July 2007 to March 2008 in Rachaburi province and during October 2007 to May 2008 in Nakhon Phatom province.

*Biology and ecology of B. dorsalis on rose apple at laboratory conditions*

Infested fruits were collected and retrieved to the laboratory, where all further studies were performed at constant conditions ( $23.10 \pm 1.27^\circ\text{C}$  and  $91.07 \pm 1.25\%$  RH).

*Study of life cycle stages of B. dorsalis.* Each developmental stage (egg, larvae, pupal and adult) was studied as follows.

Egg stage. Duration of egg stage was studied by determining hatching rate. One hundred eggs were transferred on a wet filter paper (No. 91) placed inside the Petri dish (9 cm- diam. x 2 cm-high). Egg hatching was observed every 6 hours on 5 Petri dishes containing 100 eggs each.

Larval stage. Duration of larval period and characteristics of each larval instar were studied by rearing larvae in rose apples. The information on size, characteristics and mortality on 100 larvae was recorded.

Pupal stage. Duration of pupal period and pupal characteristics were studied by observing 100 pupae.

Adult stage. Longevity, mating, fecundity and characteristics of adult fruit fly were studied by rearing a pair of *B. dorsalis* in a plastic box (21 x 15 x 8 cm.) Water and artificial diet for adult were placed inside plastic box. The polyethylene container (2.5 cm-diam x 4.5 cm-high) was used as egg receptacle. Twenty small holes were made on the side of the container. To provide the oviposition stimulus and to prevent the egg from desiccating, the inside of the egg receptacle was filled with 5 ml solution of 100 % orange juice diluted with water. The ratio of orange juice to water was 1:2. Eggs were collected and counted every day until female died. In addition, the following information was also recorded: characteristics of adult male and female, mating behavior and mortality. This study was done with 10 pairs of fruit fly.

*Determination of life table of B. dorsalis.* Five rose apple fruits were pierced, forming a small hole (1 x 1 x 1 cm) on each fruit. A piece of black filter paper (0.5 x 0.5 cm) was inserted into each hole containing 20 *B. dorsalis* eggs. Each hole was sealed with parafilm paper. Each fruit was placed in a container, determining the number of hatched eggs, number of larvae of each instar, pupae and adults.

### *Fruit fly control measures on rose apple*

*Study on the effectiveness of different fruit fly exclusion bags.* The objective was to determine the most effective plastic bags that could completely protect rose apples from fruit fly infestation. The study was separated into 2 experiments (with different number of fruits per bag Figs. 1 and 2) and carried out at laboratory conditions. For both experiments bagged rose apples and unwrapped (control) ones were exposed to ~1,000 gravid *B. dorsalis* females (~2 weeks old) in infestation cages during 24h (with ~1,000 males; Fig. 3). After exposure period, rose apples were removed from infestation cages, plastic bag removed and placed in plastic boxes (16.5 cm-diam. x 16 cm high). Isolated treated-exposed rose apples were held in room for 7 days, then dissected and visually examined for immature stages of the fruit flies. Number of infested fruits was also recorded.

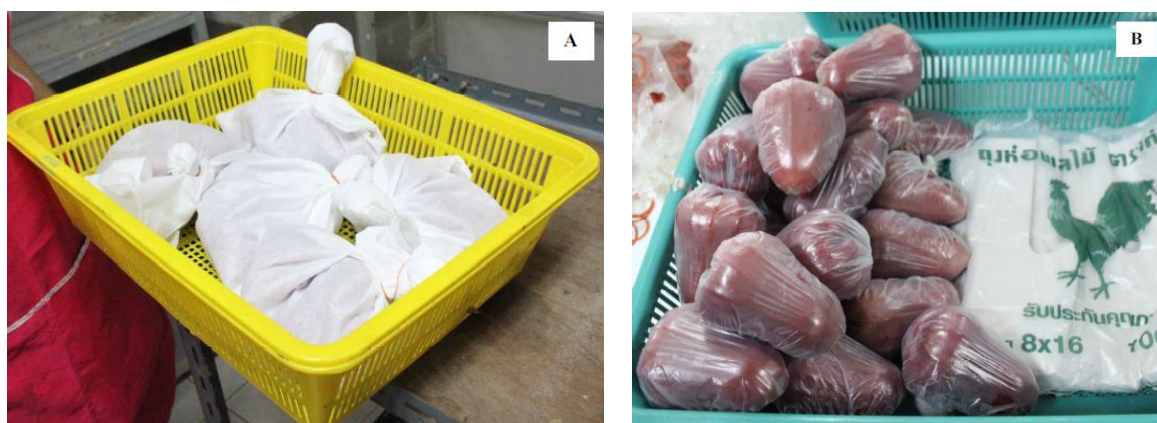
Experiment 1. Three rose apples were bagged for protection against *B. dorsalis* before exposing them to *B. dorsalis* gravid female (Fig. 1). The experiment was arranged in Completely Randomized Design (CRD) with 4 treatments and 13 replicates. Each replicate consisted of 3 rose apples held in a plastic bag. Whereas treatments consisted on: (1) White plastic bag ('Rod Tak' brand) (8 x 16 inches); (2) White plastic bag ('Ta Kai' brand) (8 x 16 inches); (3) Polyester spunbond bag (12.5 x 16.5 inches); and (4) unwrapped, to be used as control.

Experiment 2: Individually wrapped rose apples were exposed to *B. dorsalis* gravid female (Fig. 2). The experiment was arranged in CRD with the same 4 treatments as for experiment 1, but with only 10 replicates.

Both white plastic bags ('Rod Tak' brand and 'Ta Kai' brand) are high density polyethylene (HDPE) which is a polyethylene thermoplastic made from petroleum. The thickness of the plastic used to make white plastic bag ('Rod Tak' brand) and white plastic bag ('Ta Kai' brand) was approximately 40-45 microns and 30-35 microns, respectively.



**Fig. 1.** Experiment 1: 3 rose apples were held in a bag before exposing to gravid females for oviposition. A. Polyester spunbond bag (12.5 x 16.5 inches); B. White plastic bag (8 x 16 inches).



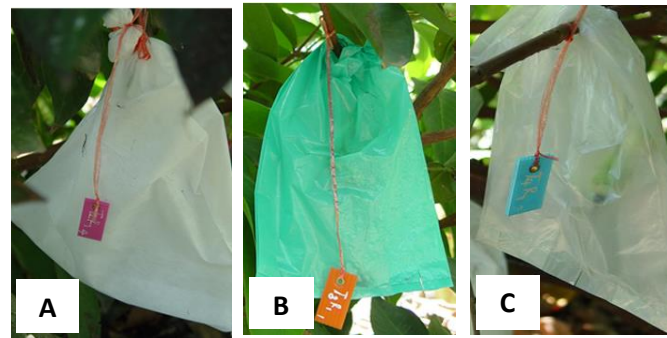
**Fig. 2.** Experiment 2: Rose apples were tightly wrapped individually by different plastic bags before exposing to gravid females for oviposition. A. Polyester spunbond bag (12.5 x 16.5 inches); B. White plastic bag (8 x 16 inches).



**Fig. 3.** Tested fruits were exposed to gravid females for oviposition for 24 hours in infestation cages.

*Comparison on different methods to control fruit flies on rose apple.* The objective of this study was to determine the most effective control method that can completely prevent rose apple from fruit fly infestation. The experiment was conducted on 2- year-old rose apples of ‘Tabtimjan’ cultivar in the orchard located at Amphur Sampan (Nakhon Pathom province). Fruits were bagged starting 14 days after stamens fell off, the number of fruits was recorded prior bagging. At harvest time, number of undamaged and damaged (with differentiation of pest species) fruits was recorded. The experiment was arranged in Randomized Complete Block Design (RBD) with 4 treatments and 4 replications. Each replication consisted in 10 clusters of inflorescence held in a bag (Fig. 4). Applied treatments were: (1) Petroleum oil (SK Enspray 99), at the rate of 60 ml in 20 liters of water, was applied for 7-day interval; (2) Polyester spunbond bag (12.5 x 16.5 inches); (3) Treatment 3: Green plastic bag (8 x 16 inches); and (4 or control) white plastic bag (7 x 15 inches).





**Fig. 4.** Ten clusters of inflorescence were held in a bag. A) Polyester spunbond bag; B) Green plastic bag; C) White plastic bag.

*Large-scale trial on a system approach for fruit fly management in rose apple orchards (IPM).* The objective of this experiment was to assess the effectiveness of a system approach for fruit fly management in rose apple orchard, which is comparable to IPM. The experiment was done on 4-year-old rose apple trees of ‘Tabtimjan’ cultivar from July to August 2012 at Amphur Sampan (Nakhon Phatom province). The proposed IPM involved: (1) field sanitation by the destruction of all unmarketable and already infested fruits, which should be buried at least 15cm in depth, to prevent the accumulation of fruit flies in the orchards; (2) Tree pruning, to create unfavorable environmental conditions for fruit flies; (3) Population monitorization and *Bactrocera* spp. male annihilation by using Methyl eugenol baited traps hanged on tree canopy at ~ 1.5 meter high (8 traps cover an area of 1,600 square meters); (4) application of fruit fly exclusion barriers at each inflorescence (flowers clutches were reduced to only 2-3 fruits) at 14 days after stamens fell off, by using white plastic bag (‘Rod Tak’ brand) (8 x 16 inches); and (5) poison protein bait treatment (chemical-baited treatment) to reduce whole fruit fly population at the orchard, applied in a weekly manner in four spots (30 x 30 cm) per tree from one week before fruit bagging (exclusion barrier) until harvest. Poison protein bait consisted on a mixture of 200 ml of protein bait (Brewer’s yeast), with 40 ml of malathion (83 % EC, Dimark) in 5 liters of water, to be applied at the evening by using a knapsack sprayer. To assess the performance of this IPM program, 100 fruits (10 fruits per tree) were randomly collected before (control) and after treatments application. Number of undamaged and damaged fruits was recorded. Weekly fruit fly captures were also identified and recorded.

## Results and Discussion

### *Survey and ecological study of economically important arthropod species on rose apples*

*Survey of fruit fly species on rose apples.* The ten surveys performed, revealed that *B. dorsalis* and *B. correcta* were found at both provinces, whereas *B. carambolae* was only found at Rachaburi province (Table 1). This result was similar to the surveys reported by Montree (1999, 2001) and Sen (1986) that found 3 or 2 *Bactrocera* species attacking rose

apples.

*Fruit fly natural enemies survey in rose apple orchards.* Two hymenopteran parasitoids were identified emerging from *Bactrocera* spp pupae (Table 1), the egg-parasitoid *Fopius arisanus* (Sonan) and the larval-pupal wasp, *Diachasmimorpha longicaudata* (Ashmead). The Rachaburi province presented the highest parasitism rates 2.99- 9.21%, and widely distributed that the Nakhon Pathom province parasitoids were found only in one survey and at a low parasitism rate (2.22 %).

**Table 1.** Number and species of fruit fly on rose apples at Rachaburi and Nakhon Pathom provinces.

Location	Trial	No. of fruits	No. of pupae	Emergence (%)	Adult (%)			Parasites (%)
					<i>B. dorsalis</i>	<i>B. correcta</i>	<i>B. carambolae</i>	
Rachaburi	1	96	1,208	100.00	3.97	96.03	0.	0
	2	36	457	90.37	61.11	33.80	0.69	4.40
	3	43	771	86.90	43.63	45.26	1.90	9.21
	4	29	339	95.87	60.00	37.01	0.00	2.99
	5	12	230	86.96	78.60	10.70	3.72	6.98
Nakhon Pathom	1	8	50	88.00	0.00	97.78	0	2.22
Pathom	2	3	36	69.44	60.00	40.00	0	0
	3	12	10	90.00	33.33	66.67	0	0
	4	18	40	92.50	59.46	40.54	0	0
	5	30	183	97.27	13.48	86.56	0	0

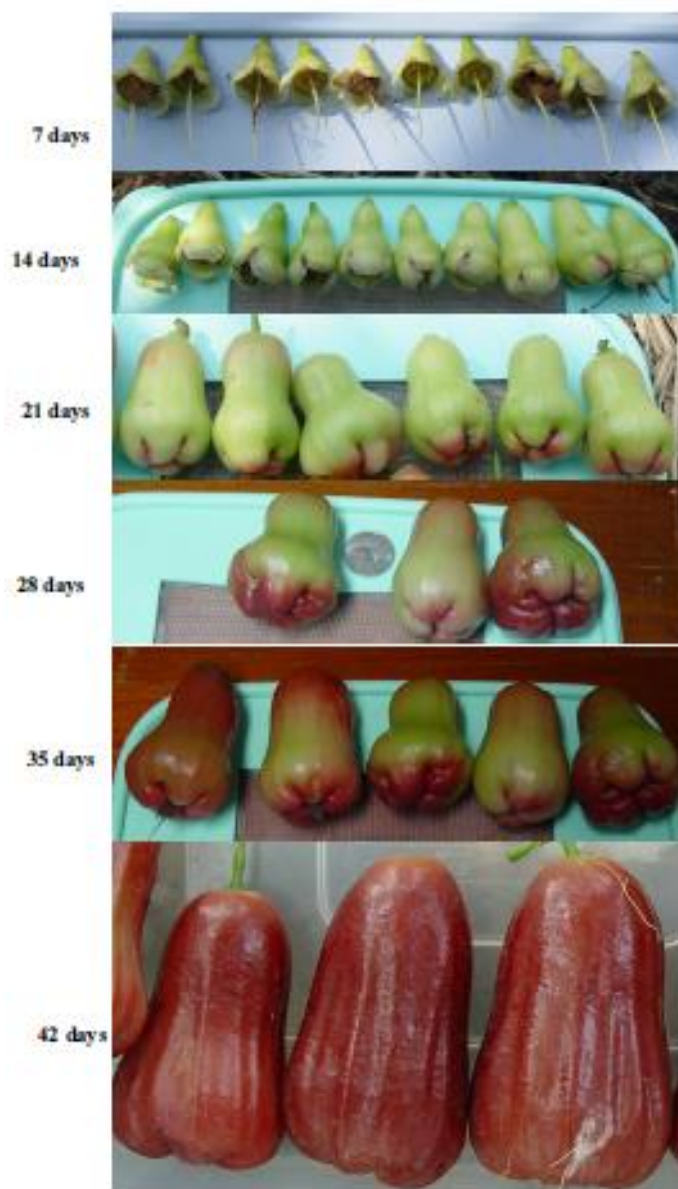
*Determination of primary pest of rose apples.* Table 2 shows average number of pupae of each species per 100 g of infested rose apple fruits. Showing that 30.73 and 24.61 belong to *B. dorsalis* and *B. correcta*, respectively. Therefore, *B. dorsalis* was considered as the primary pest of rose apples from these two provinces, deserving further research.

**Table 2.** Comparisons of number of pupae per infested fruits and number of pupae per 100 grams of infested fruits between *Bactrocera dorsalis* and *Bactrocera correcta*.

Fruit fly species	No. of infested fruits	Total weight of infested fruits (gram)	No. of pupae	No. of pupae/ infested fruits	No. of pupae/ 100 grams of infested fruits
<i>B. dorsalis</i>	5	358	110	22	30.73
<i>B. correcta</i>	3	260	64	21.33	24.61

*Rose apple pest susceptibility stages study.* Rose apple fruit development stages are presented in Fig. 5, represented in days after stamens fell-off.





**Fig. 5.** Rose Apple fruit development from 7-days to 42-days after stamens fell-off.

It was found that the number of fruit flies trapped was average 84.13, 131.00, 213.38, 155.26, 112.38, 125.25 and 195.01 flies/trap/week when the age of rose apple was 0, 7, 14, 21, 28, 35 and 42 days, respectively. Table 3 shows rose apple fruit characteristics from zero to 42 days along with results from trapped fruit flies, and percentage of fruits infested. It is noticeable the absence of fruit infestation till between fruit of 7 to 21 days-old, whereas the field traps demonstrate the presence of fruit fly peaks at the same interval. In addition to fruit fly infestation, it was found the fruit boring caterpillar, *Meridarchis* spp., also attacking rose apples, starting at 21 days after fruit set (Table 3). Montree (2009) reported also this fruit borer caterpillar as an economically important pest of rose apples. Based on the above information, the most susceptible fruit stage was set at 21 days after stamens fell-off, allowing a recommendation to

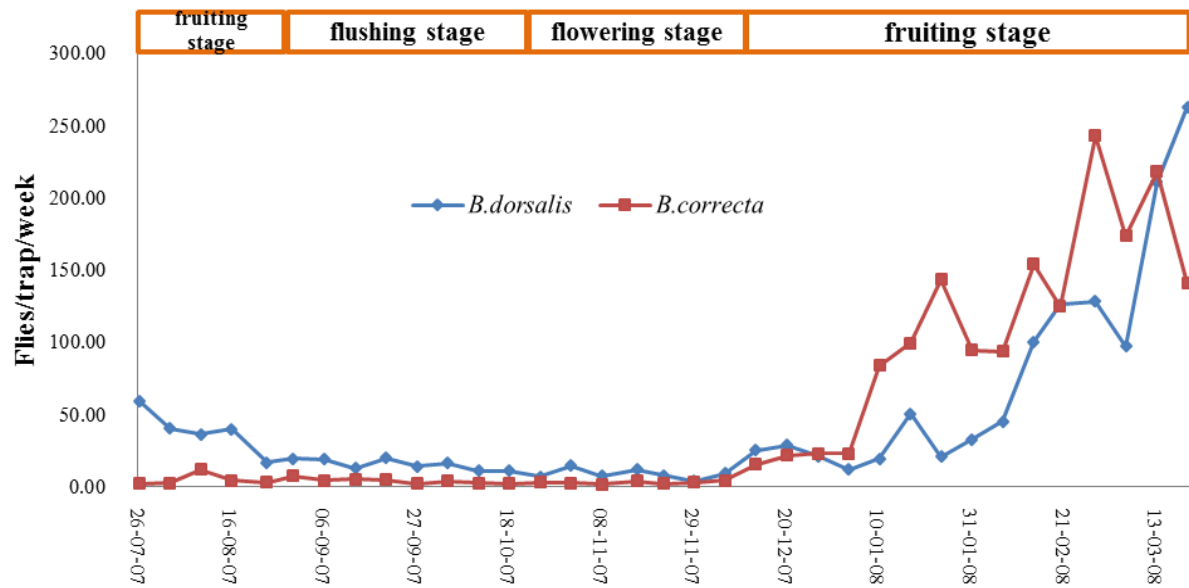
growers to protect rose apple fruits before this day, being the most suitable point at 14 days after stamens fell off to prevent the infestation of *Bactrocera spp* fruit flies and *Meridarchis spp.* caterpillar.

**Table 3.** Infestation rate of fruit borer and fruit flies on rose apples at different ages.

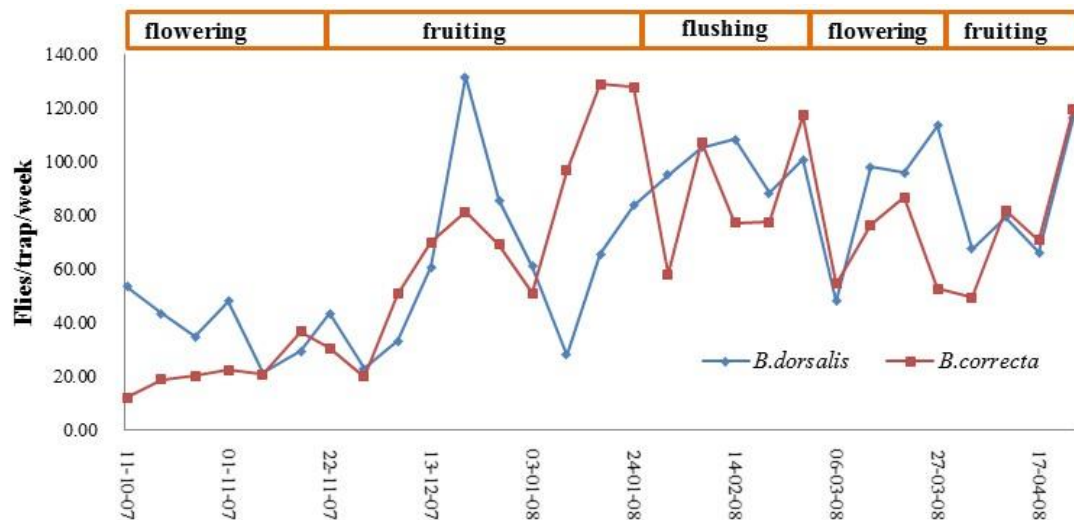
Fruit age (day)	Fruit size (cm)		Fruit weight (gram)	Infestation rate of fruit borer	Infestation rate of fruit flies	No. of fruit flies (flies/trap/w eek)
	Wide	Long		(%)	(%)	
7	1.27 ± 0.14	2.02 ± 0.09	1.79 ± 0.31	0	0	131.00
14	1.77 ± 0.19	2.67 ± 0.31	3.75 ± 1.22	0	0	213.38
21	2.92 ± 0.28	4.82 ± 0.42	17.66 ± 4.09	50	0	112.38
28	3.65 ± 0.48	5.81 ± 0.40	32.36 ± 8.18	80	30	155.26
35	4.33 ± 0.48	7.09 ± 0.36	59.44 ± 14.63	80	90	125.25
42	4.50 ± 0.34	7.87 ± 0.52	69.83 ± 19.44	100	100	195.01

*Bactrocera spp. fruit flies seasonal abundance in rose apple orchards.* Whereas the survey of infested fruits highlighted only 2 or 3 *Bactrocera* species, the trapping system set-up in rose apple orchards allowed to identify four and three species at Rachaburi and Nakhon Pathom province respectively. At the Rachaburi province were found *B. dorsalis*, *B. correcta* and *B. carambolae* despite the last two in low numbers. The seasonal abundance of *B. dorsalis* and *B. correcta* at Rachaburi province are shown in Fig. 6, having maximum peaks on March (263.25 flies/trap/week) and February (243.25 flies/trap/week) respectively (Fig. 6). In addition to these fruit flies. Whereas at Nakhon Pathom province only *B. dorsalis* and *B. correcta* were found. Their seasonal abundance is shown in Fig. 7, on which it can be seen that *B. dorsalis* peak was in December (average 131.88 flies/trap/week) and *B. correcta* peak was in late January (average 129.13 flies/trap/week), as opposite to that found in Rachaburi province (Fig. 7).

When considering fruit flies seasonal abundance and rose apple development (as indicated in Figs. 6 & 7, rose apple fruits were fully ripe and ready to start harvest from February to March and December to January), we found that fruit flies were abundant during fruit set, but their maximum population peak matched around harvest. Due to this fact, coincidence of fruit ripening with maximum fruit fly populations, growers were recommended to apply chemical treatment (pesticides) 2-3 weeks before harvest or applying protein bait before fruit fly outbreak. This helped to eliminate adult fruit flies in the orchards and also reduced fruit infestation.



**Fig. 6.** Number of *Bactrocera dorsalis* and *Bactrocera correcta* in Steiner traps collected weekly in rose apple orchard located at Amphur Damnoen Saduak, Ratchaburi province.



**Fig. 7.** Number of *Bactrocera dorsalis* and *Bactrocera correcta* in Steiner traps collected weekly in rose apple orchard located at Amphur Sampran, Nakhon Pathom province.

#### Laboratory study on biology of *B. dorsalis*

Since Sunyanee et al. (2006) already studied and reported on the biology of *B. correcta*, only the biology *B. dorsalis* was reported in this study, also lying with the determination of *B. dorsalis* as the primary pest species of rose apples. It was necessary to study the biology of *B. dorsalis* on rose apples in order to know information on its behaviors including life span,

time to infest fruits and susceptible stage. This information was used as basic information to establish appropriate control measures.

*Life cycle of B. dorsalis.* As other tephritids, *B. dorsalis* show a life cycle composed of egg, three instar larval, pupal and adult stages. The duration of each stage is shown in Table 4, whereas morphological description is presented below. This study showed that *B. dorsalis* required in average  $17.80 \pm 1.34$  days to develop from egg to adult, with a survival rate of 38%, also from egg to adult (Table 4 and Table 5).

Egg stage. Gravid female inserted the ovipositor into the fruit about 2-5 mm beneath fruit surface, 2-3 eggs were deposited in each clutch. Eggs were tiny, in average  $1.27 \pm 0.07$  mm long and  $0.21 \pm 0.02$  mm wide. They were shiny and whitish when freshly laid, but later became darker when about to hatch. Incubation period was 42-72 hours. Hatching rate was high up to average 87 %.

Larval stage. Larvae were white and transparent after hatching and darkening later in development. They were typical fruit fly shape which was cylindrical-maggot shape, elongate, anterior end narrowed and curved ventrally, with anterior brown mouth hook. They were three larval stages lasting for about 6-8 days. The 1 instar larvae were average  $1.07 \pm 0.14$  mm long and  $0.25 \pm 0.03$  mm wide. The 2 instar larvae were average  $4.88 \pm 0.34$  mm long and  $1.08 \pm 0.07$  mm wide. The 3 instar larvae were average  $7.63 \pm 0.64$  mm long and  $1.67 \pm 0.14$  mm wide. They were able to pop up for up to 30 cm. Popping helped mature larvae to find suitable places for pupation. Survival rate of larvae was average 63.22 %.

Pupal stage. Pupae were barrel-shaped, whitish at first but later became yellow-brown when near to eclosion. Pupae lived under the soil about 2-5 cm deep. They were in average  $4.71 \pm 0.17$  mm long and  $2.18 \pm 0.09$  mm wide. Pupae lasted for about 9-10 days and survival rate was in average 82.61 %.

Adult stage. Adults were reddish brown with a clear wing membrane and yellow post-pronotal lobes. Adult females became receptive to courting males at about 8 days after emergence and began to lay eggs in host fruits. Each female was capable of laying in average about  $597.29 \pm 62.38$  eggs during its lifetime, with a maximum of 40 eggs/day. Sex ratio was 1:1.36 (Female: Male). Adult females were average  $0.93 \pm 0.12$  cm long and  $1.47 \pm 0.13$  cm wide (wings span). They lasted for about 79-120 days or average  $95.03 \pm 11.87$  days. Adult males were average  $0.82 \pm 0.07$  cm long and  $1.42 \pm 0.19$  cm wide (wings span). They lasted for about 86-132 days or average  $97.50 \pm 9.31$  days (Table 4).

*Life table of B. dorsalis* was similar to that of *B. correcta* (Sunyanee et al., 2006), results are presented in Table 5. As can be seen, the first instar larvae was the most susceptible stage, whereas the second instar larvae had the higher survival rate. Similarly, *B. correcta* also presented a higher mortality rate (33.99%) for first instar and the lower for the second instar larvae (3.30%) (data from Sunyanee et al., 2006).

**Table 4.** Life cycle of *Bactrocera dorsalis* at constant temperature and humidity ( $23.10 \pm 1.27$  °C and  $91.07 \pm 1.25$  % RH).

Stage	No. of tested	Duration	
	individuals	(day)	Mean $\pm$ SD
Egg	100	42 - 72 (hours)	48.96 $\pm$ 10.88 (hours)
Larva	100	6 - 8	6.07 $\pm$ 0.30
Pupa	100	9 - 10	9.21 $\pm$ 0.41
Adult			
Female	10	79 - 120	95.03 $\pm$ 11.87
Male	10	86 - 132	97.50 $\pm$ 9.31
Duration from egg to adult		16.75 - 20.75	17.80 $\pm$ 1.34

**Table 5.** Life table of *Bactrocera dorsalis* in rose apples.

Stage (x)	$l_x$	$L_x$	$d_x$	$100q_x$	$S_x$	$e_x$
Egg	100	93.50	13	13.00	87.00	3.17
Larva						
1 <sup>st</sup> - instar	87	73.50	27	31.03	68.97	2.57
2 <sup>nd</sup> - instar	60	57.50	5	8.33	91.67	2.50
3 <sup>rd</sup> - instar	55	50.50	9	16.36	83.64	1.68
Pupa	46	42.00	8	17.39	82.61	0.91
Adult	38	-	-	-	-	-

x = development stage

$l_x$  = number of live individuals in stage x

$L_x$  = average number of live individuals in each development stage

$d_x$  = number of dead individuals in stage x

$100q_x$  = percentage of mortality rate in each stage

$S_x$  = survival rate in each development stage

$e_x$  = expected values of survival during each development stage

### *Fruit fly control measures on rose apple*

*Study on the effectiveness of different fruit fly exclusion bags.* Tables 6 and 7 shown the results of the two experiments performed to determine the success of plastic bags as barrier for fruit flies. In both experiments, control fruits were 100% infested, whereas the other three treatments (plastic types) showed differential results depending on how many fruits were located within each bag (experiment 1 with three fruits per bag, and experiment 2 only one fruit per bag). The results for this study indicated that rose apples wrapped with different plastic bags response differently to fruit fly infestation. It obviously showed that the thickness of plastic play an important role in determining the effectiveness of the bags as fruit fly barrier on rose apples. The white plastic bag ('Rod Tak' brand) which is approximately 40-45 microns thick provided better fruit protection than white plastic bag ('Ta Kai' brand) and polyester spunbond bag. Therefore, the use of a white plastic bag which is over 40 microns thick is recommended to bag rose apples to prevent fruit fly infestation.

**Table 6.** Experiment 1<sup>1</sup>: Infestation rate of fruit flies on rose apples wrapped with different plastic bags.

Treatment	Tested fruits (n)	Infested fruits (n)	Infestation rate (%)
White plastic bag ('Rod Tak' brand)	150	0	0.00
White plastic bag ('Ta Kai' brand)	150	23	15.33
Polyester spunbond bag	150	0	0.00
Control (Unwrapped)	150	150	100.00

<sup>1</sup>3 tested fruits were held in a plastic bag before exposing to gravid females for oviposition.

**Table 7.** Experiment 2<sup>1</sup>: Infestation rate of fruit flies on rose apples wrapped with different plastic bags.

Treatment	Tested fruits (n)	Infested fruits (n)	Infestation rate (%)
White plastic bag ('Rod Tak' brand)	80	0	0.00
White plastic bag ('Ta Kai' brand)	80	12	15.00
Polyester spunbond bag	80	2	2.50
Control (Unwrapped)	80	80	100.00

<sup>1</sup> Each tested fruit was tightly wrapped with plastic bag before exposing to gravid females for oviposition.

*Comparison on different methods to control fruit flies on rose apple.* Prior to the experiment, number of fruits for all treatments was not statistically different. However, number of fruits after completion of the experiment was statistically different. Polyester spunbond bag provided the best fruit protection. Average number of fruits was 3.43 fruits for Treatment 2 (polyester spunbond bag) which was not statistically different from Treatment 3 (white plastic bag) but statistically different from Treatment 4 (green plastic bag) and Treatment 1 (petroleum oil). Average number of fruits was only 0.03 fruit for treatment of petroleum oil which was statistically different from other treatments (Table 8). Quality of rose apples at harvest is shown in Fig. 8.



**Fig.8.** Quality of rose apples in different bags. A. Polyester spunbond bag; B. White plastic bag.

**Table 8.** Number of fruits and fruit weight of rose apples after subjecting to different control measures for fruit flies.

Treatment	No. of fruits		Fruit weight (gram)	Damaged fruits caused by fruit flies (%)	Damaged fruits caused by fruit rot (%)
	Before bagging	After completion of the experiment			
Petroleum oil	4.25	0.03 c <sup>1/</sup>	1.25 c <sup>1/</sup>	99.41	0
Polyester spunbond bag	4.35	3.43 a	77.12 ab	0	21.84
Green plastic bag	4.25	1.58 b	56.50 b	0	62.94
White plastic bag (Control)	4.25	3.05 a	86.09 a	8.11	20.13
CV (%)	8.10	28.90	30.00	-	

<sup>1/</sup> Values are mean of 4 replications. In column, means followed by a common letter are not significantly different at 5% level by DMRT.

Weight of fruits for white plastic bag was the highest average 86.09 grams which was not statistically different from polyester spunbond bag but statistically different from green plastic bag and petroleum oil. Weight of fruits for petroleum oil was the lowest average 1.25 grams which was statistically different from other treatments (Table 8).

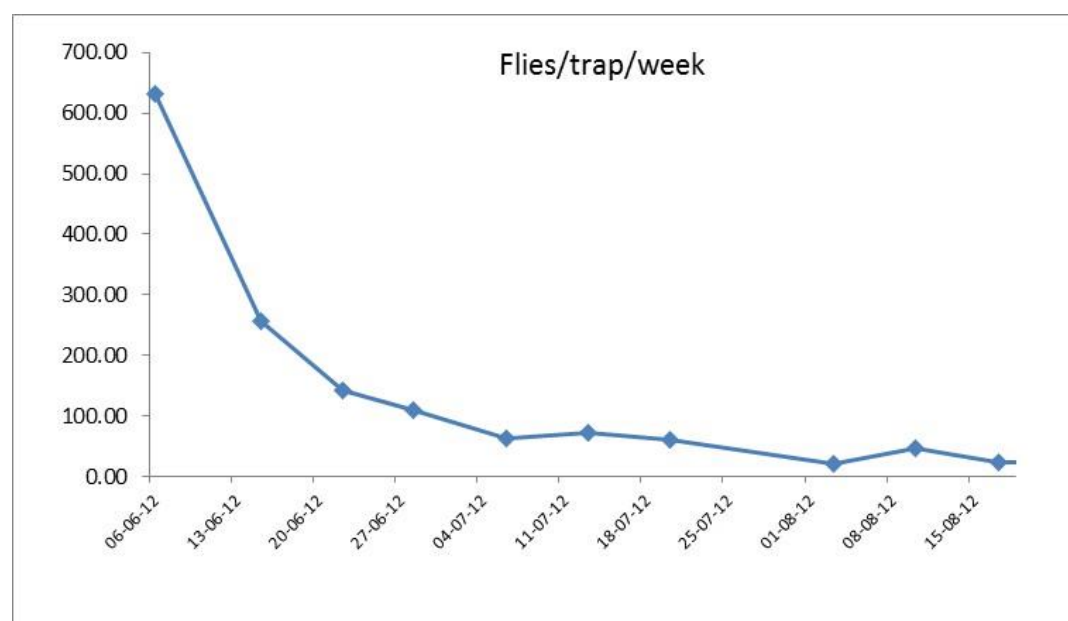
No fruit fly infestation was found on fruits bagged with polyester spunbond bag and green plastic bag (Table 8), whereas fruits sprayed with petroleum oil and bagged with white plastic bag were infested 99.41 and 8.11 %, respectively. Although both polyester spunbond bag and green plastic bag could protect fruits from fruit fly infestation, fruits bagged with green plastic bag show higher infection rate of fruit rot than fruits bagged with polyester spunbond bag. Therefore, green plastic bag was not recommended to use as a fruit fly exclusion bag for rose apple.

Results from the experiment indicated that polyester spunbond bag tended to provide better protection than white plastic bag which growers commonly used. Generally, growers used white plastic bag to bag 2-3 fruits and some fruits were discarded before bagging. For polyester spunbond bag, all fruits were bagged. It was observed that each inflorescence could bear fruits up to 8 fruits. Therefore, using polyester spunbond bag tended to be effective because large number of fruits could be bagged.

*Large-scale trial on a system approach for fruit fly management in rose apple orchards (IPM).* Fruit fly population tended to decrease after the application of fruit fly management in rose apple orchard. Before initiation of the trial, the number of fruit flies trapped was average 626.56 flies/trap/week. During the trial, the number of fruit flies declined as showed in Fig. 9. Yield comparison between before and after the application of a system approach for fruit fly management showed that the number of infested fruits tended to decrease (Table 9). Fruit quality at harvest was showed in Fig. 10.

**Table 9.** Yield comparison before and after the implementation of a system approach of fruit fly management in rose apple orchards at Amphur Sampran, Nakhon Pathom province.

	Marketable fruits (n)	Fruit fly infested fruits (n)	Fruit borers infested fruits (n)	Rot fruit damaged fruits (n)
Before implementation	77	13	7	3
After implementation	95	2	2	1



**Fig. 9.** Number of fruit flies in Steiner traps collected weekly in rose apple orchard at Amphur Sampran, Nakhon Pathom province after the implementation of a system approach.

Based on the preliminary study, the use of a system approach was significantly reduced fruit fly infestation on rose apples. A system approach for fruit fly management consisted of 5 major components including (1) orchard sanitation (2) pruning trees (3) methyl eugenol trap (4) bagging 3 fruits/bag with white plastic bag (8 x 16 inches) and (5) spraying of poison protein bait. This system approach will be implemented as a risk management of fruit flies for exporting rose apples from Thailand to countries which have serious phytosanitary import requirements.





**Fig. 10.** Quality of rose apples from the orchards which implemented a system approach for fruit fly management.

## Conclusion

Three tephritid species found in Thailand rose apple orchards (*B. dorsalis*, *B. correcta* and *B. carambolae*), only three were found infesting the rose apple fruits, and only *B. dorsalis* was considered as a primary key pest, lasting in average ~18 days to develop from egg to adults in rose apples. Two fruit flies natural enemies, the hymenopteran parasitoids *D. longicaudata* and *F. arisanus* were also found in rose apple orchards, inducing a low control of fruit flies, a factor that deserves further research.

In this study we determined that rose apple ripening coincided with the highest *Bactrocera* spp. population peaks. The following IPM measures were determined as the best suited for *Bactrocera* spp. population reduction while maintaining low fruit damage and low cost: (1) orchard sanitation; (2) tree pruning; (3) use of methyl eugenol traps for monitorization and male suppression; (4) use of fruit fly exclusion bags at 14-days after stamens drop (a white plastic of ~ 40-45 microns and 8x16 inches per each 3 fruits cluster); and (5) limitation to one chemical treatment at pre-harvest.

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## Mass trapping for *Anastrepha suspensa*

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### Abstract

**Background:** Mass trapping has been found to be highly effective for control of pest fruit flies when populations are low and a highly effective lure is available for the target species. Successful population control through mass trapping is an indicator that attract-and-kill bait stations may be equally successful, and can provide a predictor of levels of control that can be achieved through use of bait stations. The Caribbean fruit fly, *Anastrepha suspensa* (Loew), is a serious pest of guava, *Psidium guajava* L., and growers in south Florida, USA, are in need of alternatives to pesticide application for fruit fly control.

**Methods:** Field studies were conducted in a small planting of guava to investigate the potential use of mass trapping for control of *Anastrepha suspensa*. Multilure traps baited with a two component food-base lure comprised of ammonium acetate and putrescine (BioLure) were placed in every tree throughout the season. Numbers of male and female flies trapped were determined weekly by placing Multilure traps baited with torula yeast/borax solutions in 5 trees in the middle of plots with mass traps and without mass traps (control) for 24 h. Additional data were collected on number of flies per mass trap and fruit infestation levels.

**Results:** Results of tests conducted over several years found that mass trapping had the potential to not only decrease number of adult flies in the plot but also to decrease infestation level during some of the sample periods.

**Conclusions:** Although these tests were conducted in areas that are not optimal for control, mass trapping was found to be successful. Deployment over a larger area and development of more effective attractants for this pest may provide a new tool for guava growers in south Florida.

**Keywords:** *Anastrepha suspensa*, guava, synthetic lures, torula yeast.

### Introduction

Mass trapping is a pest control option that involves deploying a large number of traps to remove pest insects and suppress the pest population. Early studies conducted in Mexico found that McPhail traps baited with aqueous cottonseed hydrolysate and borax solution, deployed in alternate trees in citrus or with 1-4 traps per tree in mango (*Mangifera indica* L.), were effective in reducing adult *Anastrepha ludens* (Loew) populations and fruit infestations early in the season when fly populations were low (Balock & Lopez, 1969). However control was lost when populations increased to high levels later in the season. Other studies have also

shown mass trapping to be effective for control of pest fruit flies when populations are low, and a highly effective, female-targeted lure is available (El-Sayed et al., 2006). Mass trapping with traps baited with food-based synthetic attractants has been used successfully for control of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), in Spain (e.g., Leza et al., 2008; Navarro-Llopis et al., 2008; Martinez-Ferrer et al., 2012). The Caribbean fruit fly, *Anastrepha suspensa* (Loew), is a serious pest of guava, *Psidium guajava* L., in south Florida, USA, and growers are in need of alternatives to pesticide application for fruit fly control. The objective of this work was to investigate the potential use of mass trapping for control of *A. suspensa* in field studies conducted in a small planting of guava.

## Material and Methods

### *Traps and Lures*

MultiLure traps (Better World Manufacturing Inc., Fresno, CA, USA) were used in all experiments. Traps used for mass trapping adults were baited with a two component synthetic food-based attractant (Biolure formulation of ammonium acetate and putrescine lures; Suterra LLC, Bend, OR, USA). This lure has been found to be the most effective attractant for *A. suspensa* (Epsky et al., 2011). Mass traps also contained 300 ml 10% polypropylene glycol (vol:vol; LowTox, Prestone, Danbury, CT, USA) aqueous solution to retain captured flies. Mass traps were sampled weekly and lures were replaced after 4 or 5 wk of continuous trapping. Traps used for monitoring adults were baited with aqueous solutions of torula yeast/borax (three 5-g pellets, 2.25:2.75 yeast: borax, in 300 ml water; ERA International, Freeport, NY, USA) and were placed in 5 central trees (1 in the middle tree and 4 in diagonally-located trees adjacent to the middle tree) in both mass trap and untreated (control) blocks. Each week, monitoring traps were placed in the field for a 24 h period, sampled and removed from the field. Fresh aqueous protein bait was used each week. Flies collected from both mass traps (7 d time period) and monitoring traps (1 d time period), were transferred to aqueous ethanol (70% v/v), and numbers of male and female flies per trap were recorded. Location of each mass trap within the grid was recorded to facilitate spatial analysis. The distribution of adults in the mass trapping block was evaluated by spatial analysis using Surfer 8.05 (Golden Software, Inc., Golden, CO, USA) to generate contour maps of the females per trap per week. Separate contour maps were produced for data from each week of the study and were examined visually.

### *Field Test*

Field tests were conducted at the University of Florida Tropical Research and Education Center in Homestead, FL, USA. Guava trees used for these tests had been planted in a 5 by 30 tree grid between 15 Sept 1995 and 25 June 1997, and tree spacing was 6.1 m by 6.1 m within the grid (0.56 ha). There were 35 trees per block (5 trees by 7 trees). Untreated (control) blocks contained 5 monitoring traps only; mass trap blocks had one mass trap per tree and 5 monitoring traps. Treatments were replicated over time and mass traps were placed in the

field prior to the start of the fruiting season, when adult fly populations were low and there was no fruit available for reproduction within the guava planting. Guava trees began fruiting earlier in 2008 than in 2009 and 2010 because the trees were trimmed in spring 2009 and 2010. The trimming shortened the fruiting season, but increased the amount of fruit on the trees during the fruiting season. The experiment was divided into three time periods in 2008, and treatments were rotated sequentially among three blocks at the start of each time period: 16 April to 10 June (8 wk), 10 June to 6 August (9 wk), and 6 August to 31 October (8 wk). Treatments were not rotated in the 2009 and 2010 studies, but remained in the same block throughout the study. This change was made to provide season-long treatment, which replicated the control approach that would be used by growers. Tests were conducted from 30 July to 27 August (4 wk), 27 August to 30 September (5 wk), 30 September to 4 Nov 2009 (5 wk), and 16 July to 15 Sept 2010 (9 wk). There was a total of 7 replicate time periods.

#### *Fruit availability and infestation level*

Fruit availability and fruit infestation levels were monitored periodically throughout the study. Fruit availability was determined by counting the number of fruit on each tree that ranged from being large enough for oviposition (dark green, golf ball size) to ripe (light green to yellow) (fruit growth stage to fruit ripening stage [Salazar et al., 2006]). Number of fruit was converted to an abundance rating (0 = no fruit, 1 = 1-5 fruit, 2 = 6-10 fruit, 3 = 11-15 fruit, 4 = 16-20 fruit, and 5 > 20 fruit) for analysis. Fruit availability was assessed 20 May, 2 July, 5 August, 10 September, 14 November 2008; 5 August, 23 September, 4 November 2009; 30 July and 15 September 2010. Fruit infestation was determined for ten fruit per block, which were selected randomly from trees located in the center of the block. Fruit were weighed, held individually for 6 wk and number of pupae per fruit determined. Fruit infestation was assessed 28 May, 9 July, 5 August, 3 September, 1 October 2008; 19 August, 23 September, 4 November, December 3 2009; and 17 September 2010.

#### *Statistical Analysis*

Two sample *t*-tests using Proc TTEST (SAS Institute 2010) were used to compare numbers of flies per monitoring trap per day, fruit availability and fruit infestation between untreated and mass trapping blocks. Proc TTEST uses the folded F method to determine equality of variance between treatments, and *t* value determined using the Satterthwaite method was used to determine the appropriate degrees of freedom when the variance was unequal.

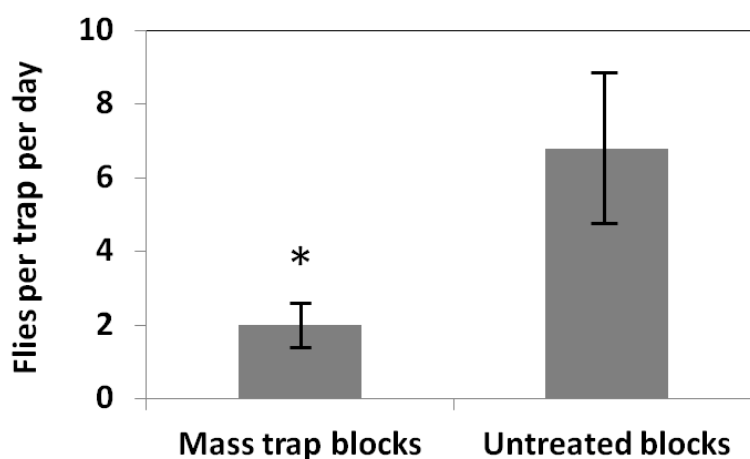
## Results

### *Number of adults in monitoring traps*

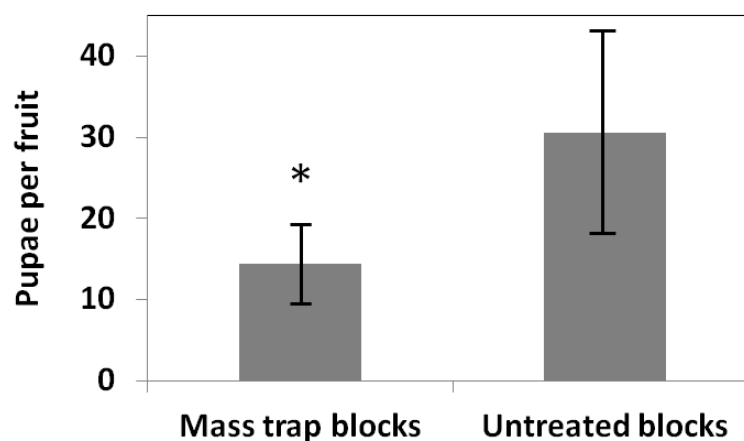
More *A. suspensa* were captured in monitoring traps in the untreated plots than in the plots with the mass traps ( $t = 2.37$ ,  $df = 6.7$ ,  $P = 0.05$ ; Fig. 1). Among the 7 replicates, differences ranged from 0 - 12 flies per trap per day in untreated versus mass trap blocks.

### *Fruit availability and infestation level*

Although there were mixed varieties of guava trees in each plot, there was no difference in fruit availability between mass trap plots ( $2.3 \pm 1.3$ ) and untreated plots ( $2.4 \pm 1.3$ ). However, untreated plots had higher levels of larval infestation than mass trap plots ( $t = 2.17$ ,  $df = 11.8$ ,  $P = 0.0479$ ; Fig. 2). Among the 12 replicates, differences ranged from 0 - 72 pupae per fruit in samples obtained from untreated versus mass trap blocks.



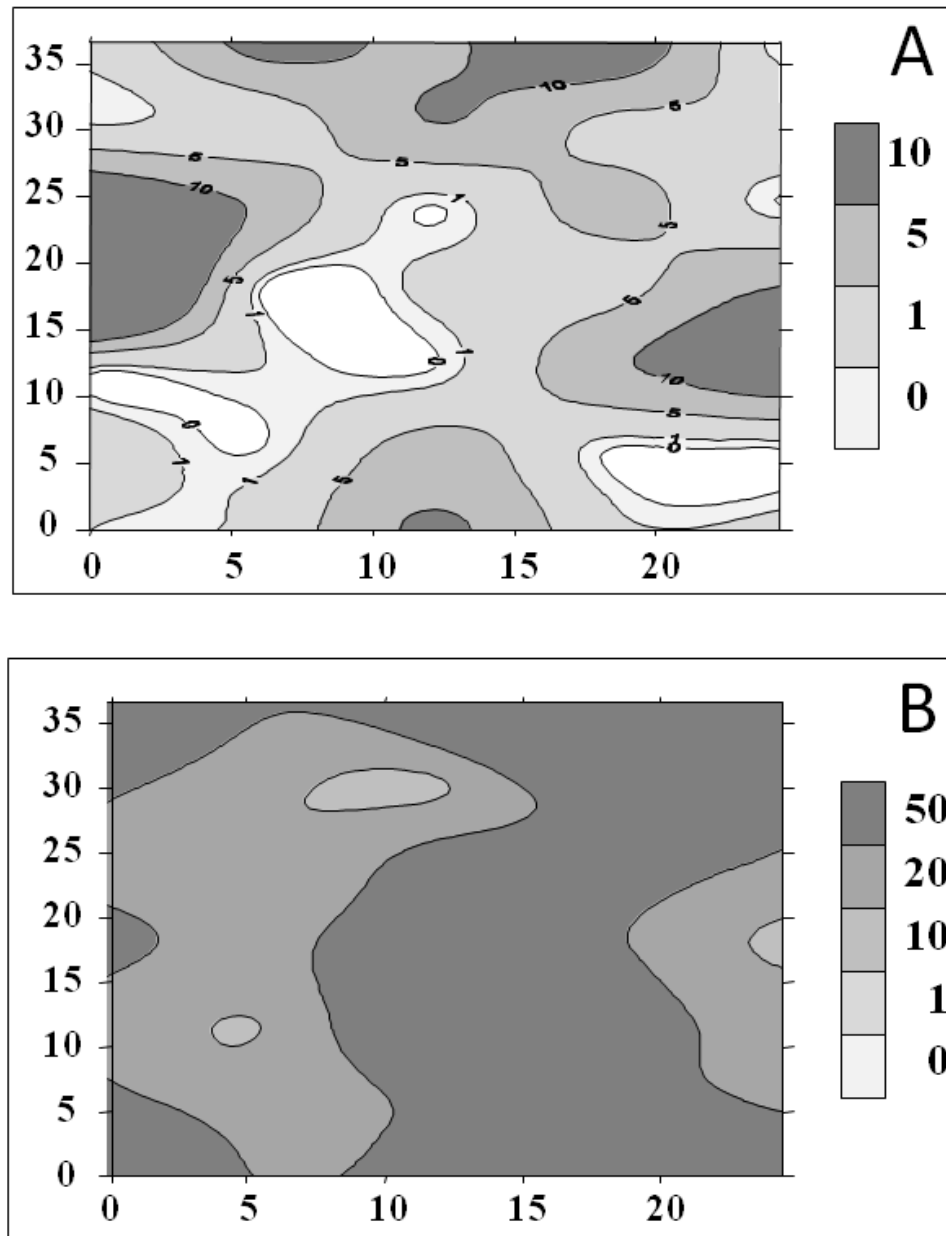
**Fig. 1.** Average (SE) number of adult *A. suspensa* per trap per day captured in monitoring traps baited with aqueous torula yeast/borax solution.



**Fig. 2.** Average (SE) infestation of *A. suspensa* per fruit in mass trap versus untreated blocks.

*Spatial analysis*

Contour maps of distribution of females captured in mass traps indicated that early in the season, when populations were low, mass traps intercepted females as they entered the field, protecting the center of the plot (Fig. 3A). Later in the season, when populations were high, flies were found throughout the field and the population was no longer suppressed (Fig. 3B).



**Fig. 3.** Representative examples of the distribution of female *A. suspensa* captured per trap per week in mass traps A) early in the season when populations were low (0-10 females per trap) and B) in mid-season when populations were high (0-50 females per trap).

## Discussion

Mass trapping has been shown to be promising for not only tephritids (Navarro-Llopis & Vacas, 2014), but also for a wide range of pests including blood-feeding flies (e.g., Kline 2007) and beetles (e.g., Schlyter et al., 2001; Alpizar et al., 2002). Mating disruption is more widely used for moths, although identification and availability of female-targeted pheromones, kairomones or other food-based lures will increase mass trapping options for lepidopteran pests (Witzgall et al., 2008).

In our study, mass trapping decreased populations of *A. suspensa* in guava, as indicated not only by monitoring traps placed in the center of the plot, but also by infestation level as determined from fruit sampling. Success of mass trapping in this system was surprising due to 1) the small size of the treated area, 2) the presence of a preferred and highly susceptible host fruit, and 3) the moderate effectiveness of the synthetic lure used to bait the mass traps. These and other limitations to efficacy of mass trapping have been reviewed by Navarro-Llopis & Vacas (2014). As was observed in early studies of *A. ludens* with liquid protein-baited mass traps (Balock & Lopez, 1969), this method was most effective early in the season when populations were low and most of the flies were trapped prior to entering the field. Although there is some promise with this approach, it is not cost effective due to the price of the trap and lure, and the number of traps needed per hectare. However, these results indicate that mass trapping as an area-wide approach in a less preferred host fruit (e.g. citrus) may be an effective control measure for *A. suspensa*. Development of improved attractants that could reduce number needed per hectare and/or availability of inexpensive bait stations could provide a cost effective IPM tool for population suppression and/or control of this pest.

## Acknowledgements

We thank Micah Gill, Teri Allen, Wayne Montgomery, Ingris Filpo, Jeffrey Tefel, Jorge Sanchez (USDA/ARS, Miami, FL, USA) for technical assistance; Drs. Jonathan Crane (UF-TREC, Homestead, FL, USA) for access to the field site, Abbie Fox (USDA/APHIS, Palmetto, FL), Robert Meagher (USDA/ARS, Gainesville, FL, USA), Catharine Mannion (UF-TREC, Homestead, FL USA) and anonymous reviewers for comments on earlier versions of this manuscript. This paper reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation by the USDA for its use.

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## Use of male annihilation technique for control of pest species in the *Bactrocera* group on Mainland Africa

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### Abstract

**Background:** Fruit fly pest species in the *Bactrocera* group, particularly those responding to the male attractant methyl eugenol, can be effectively suppressed by the male annihilation technique (MAT). In MAT, the male fruit flies are targeted through deployment of stations or substrates containing a mixture of male attractant and an insecticide. The use of MAT on mainland Africa for suppression of recently introduced methyl-eugenol responding *Bactrocera* pest species is increasingly being considered and used.

**Methods:** The efficacy of different male annihilation methods for *B. dorsalis* control: wooden fibre board blocks impregnated with methyl eugenol and malathion, Specialised Pheromone and Lure Application Technology (SPLAT) containing methyl eugenol and spinosad and gel containing methyl eugenol and permethrin was investigated in three separate trials in commercial fruit orchards in a northern region of South Africa where *B. dorsalis* is present. In two of the trials, the male annihilation methods were in combination with application of protein-based bait stations.

**Results and Conclusions:** The results on the field studies on MAT suggested that a combination of MAT and bait application can reduce adult *B. dorsalis* catches in commercial orchards and can prevent fruit infestation. There were no major differences in the efficacy of control between the different types of male annihilation treatments. The use of MAT for control of *Bactrocera* pest species in Africa is expensive given that the parapheromones have to be imported. As such, only effective and affordable male annihilation methods for control of *Bactrocera* pest species will eventually become more widely used on the continent.

**Keywords:** *Bactrocera*, parapheromones, male annihilation technique.

### Introduction

*Bactrocera* is a genus originating mainly from the Indo-Australasian region and consisting of over 500 species (White and Elson-Harris 1994, White 2006). Several species, notably in the *Bactrocera dorsalis* complex, are regarded as some of the most destructive insects of fruit and vegetables world-wide (White and Elson-Harris 1994, Clarke et al. 2005). In Africa, only 11 indigenous species of *Bactrocera* are known, and of these only one, *B. oleae* (Rossi) is of economic importance, being a pest of cultivated olives (White 2006). However, four Asian *Bactrocera* pest species: *B. cucurbitae* (Coquillett), *B. latifrons* (Hendel), *B. zonata*

(Saunders) and *B. dorsalis* (Hendel) were introduced in Africa (White 2006, De Meyer et al. 2014). Of these, *B. dorsalis* is currently the most widespread species on the African continent (De Meyer et al. 2014) and poses the biggest threat to horticulture in Africa.

*Bactrocera dorsalis*, Oriental fruit fly, was first found in Kenya in 2003 (Lux et al. 2003) and was initially described as a new species, *Bactrocera invadens* Drew, Tsuruta and White (Drew et al. 2005). Recently, *B. invadens* was synonymised with *B. dorsalis* (Schutze et al. 2015). Since its discovery in Kenya, *B. dorsalis* was reported in many countries in Eastern, Central, Western and Southern Africa (Khamis et al. 2009, De Meyer et al. 2010). *Bactrocera dorsalis* was also recently declared present in the north/north eastern parts of South Africa (Manrakhan et al. 2015). *Bactrocera dorsalis* is a highly polyphagous species (White and Elson-Harris 1994, Clarke et al. 2005). Before the arrival of *B. dorsalis* in Kenya, the indigenous marula fruit fly, *Ceratitis cosyra* (Walker), was the predominant fruit fly pest of mango (Ekesi et al. 2009). Within four years of invasion, *B. dorsalis* displaced *C. cosyra* and became the predominant fruit fly pest of mango. In many East and West African countries, *B. dorsalis* is now the dominant fruit fly pest species attacking commercial fruits (Mwatawala et al. 2006, Ekesi et al. 2009, Vayssieres et al. 2009a, Vayssieres et al. 2010). Moreover, trade of fresh commercial fruit in many African countries has been hampered with export bans and restrictions due to the presence of *B. dorsalis* (Ekesi 2010).

The suppression tools recommended for fruit flies, including *B. dorsalis* in Africa, are application of poisoned protein baits (sprays or stations), male annihilation technique, biological control, orchard sanitation and mechanical control (bagging) (Ekesi et al. 2007). The Sterile Insect Technique (SIT) targeting *Ceratitis capitata* (Wiedemann) is another suppression tool which has been used in some regions of the Western Cape Province of South Africa (Barnes et al. 2015). Among the control tools suggested for *B. dorsalis*, a large proportion of the research focused on bait application and biological control, the most used techniques for fruit fly control in many sub-Saharan countries (Mele et al. 2007, Vayssieres et al. 2009b, Mohamed et al. 2010, Ekesi et al. 2011, Umeh and Onukwu 2011, Ekesi et al. 2014). Poisoned protein baits and some biological control agents are not specific to one fruit fly species but rather target a broader range of fruit fly pests, and this possibly explains the greater focus of research on these two techniques.

The male annihilation technique (MAT) is a behavioural manipulation method that has been successfully used in the suppression and eradication of a number fruit fly pest species, particularly in the *Bactrocera* group (Steiner and Lee 1955, Steiner et al. 1970, Qureshi et al. 1981, Calkins et al. 1984, Koyama et al. 1984, Cunningham 1989b, Fay et al. 1997, Foster and Harris 1997, Seewooruthun et al. 2000, Allwood et al. 2002, Lloyd et al. 2010, Manrakhan et al. 2011, Vargas et al. 2014). The term “Male Annihilation” was first coined by Steiner and Lee (1955) and involves the use of male lures as long distance stimuli (Cunningham 1989a, Foster and Harris 1997) in combination with an insecticide. In MAT, the mixture is incorporated in a carrier (gel or an absorbent surface like a wooden block). MAT carriers are deployed at a high density to effect high mortality of fruit fly males which would

then cause population suppression through a reduction in the number of matings and production of fertile offspring (Cunningham 1989b). MAT, unlike bait application, targets specific fruit fly pest groups which respond to the male lures being used. There are four fruit fly male lures that have been evaluated for use or are currently being used in MAT. These are methyl eugenol (ME), cue-lure, trimedlure and enriched ginger root oil, which target different fruit fly groups (Cunningham 1989a, Shelly and Pahio 2002). Of these four lures, ME and cue-lure have been more widely used for control of fruit fly pests worldwide due to the higher fruit fly responses elicited by these lures compared to other male lures (Cunningham 1989a). MAT has been used either alone or in combination with other fruit fly control techniques in various fruit fly suppression and eradication programmes. *Bactrocera dorsalis* was successfully eradicated from the Okinawa islands, Japan, in 1982 after almost 5 years of a control programme involving only aerial and ground application of MAT with ME and the organophosphate naled (Koyama et al. 1984). In 1965, *B. dorsalis* was eradicated from Mariana Islands using MAT and a combination of other techniques, including the SIT, prior to the application of MAT (Steiner et al. 1970). From the mid-1990s, MAT in combination with application of poisoned protein baits and other cultural control practices was successfully used in the eradication of *Bactrocera* species, including *B. dorsalis* (Fay et al. 1997, Seewooruthun et al. 2000, Allwood et al. 2002, Manrakhan et al. 2011). The combination of MAT and application of poisoned protein baits or the bait application technique forms the core of a number of proposed eradication methods, in particular for ME-responding *Bactrocera* pests (IAEA 2000, Manrakhan et al. 2012).

Recently, there has been an increase in the use of MAT for control of *B. dorsalis* in Africa. In this paper, we aim to (1) review the status of MAT for control of *Bactrocera* pest species in Africa, (2) provide information on ongoing research on the efficacy of male annihilation methods for control of *B. dorsalis* and (3) discuss the future perspectives, knowledge gaps and research needs on the use of MAT in Africa.

### **The use of MAT for control of *Bactrocera* species in Africa: Current status**

The first report of the use of MAT for control of an introduced *Bactrocera* pest, *B. zonata*, on the African continent was in 2001 in Sinai, Egypt (Cayol et al. 2002).

MAT was successfully used in the eradication of *B. dorsalis* on the Indian Ocean island of Mauritius, and it has also been used for area-wide suppression of *B. zonata* (Sookar et al. 2008). In 2010, MAT in combination with bait application was used for eradication of *B. dorsalis* in South Africa (Manrakhan et al. 2011). Subsequent *B. dorsalis* eradication campaigns in South Africa also involved the use of MAT and bait application (Manrakhan and Hattingh 2012, 2013). In research conducted in Kenya by Grout and Stephen (2013), the use of MAT alone for control of *B. dorsalis* was found to be less effective than a combination of MAT and bait application (either sprays or bait stations). In South Africa currently, various types of male annihilation products were registered and are available for control of *B.*

*dorsalis*. Some of these male annihilation products are being evaluated in South Africa and other African countries for control of *B. dorsalis* and results stemming from these studies will possibly increase the use of MAT on the continent.

Although MAT products are commercially available within Africa, the use of MAT across the African continent for regular suppression of *B. dorsalis* in fruit production areas is still fairly limited. In a study on practices for an integrated management of fruit fly pests in mango production areas of Embu, Kenya, Korir et al. (2015) reported that only 18% of farmers adopted MAT as a fruit fly control strategy as opposed to 47% of farmers who adopted orchard sanitation. It is likely that the limited use of MAT in Africa is attributed to the prohibitive costs of ME-based MAT products for many subsistence and small-scale fruit producers.

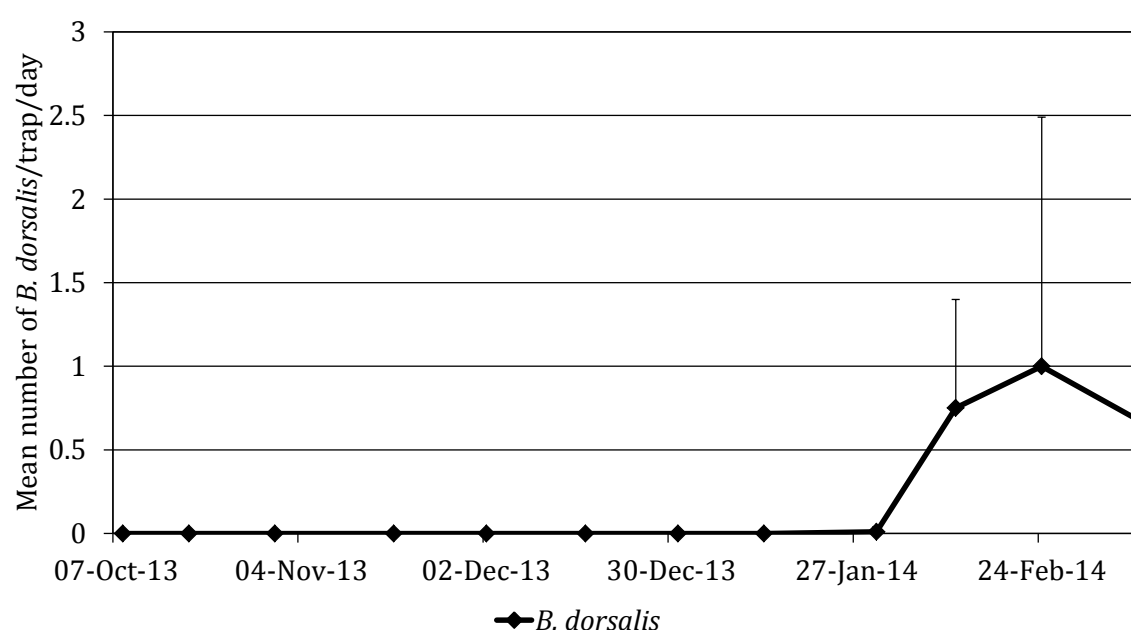
## **Research and Evaluation of MAT in South Africa**

### *Efficacy of different MAT methods in commercial orchards*

During the 2013/14 mango season the performance of different male annihilation treatments in combination with the M3 fruit fly bait stations (Green Trading cc, Brits, South Africa) for control of *B. dorsalis* was evaluated in a farm in Constantia, Limpopo Province, South Africa. Four male annihilation treatments were tested: 1) B.I. Toolkit® (Insect Science (Pty) Ltd, Tzaneen, South Africa), 2) Last Call™ B.I. (Insect Science (Pty) Ltd, Tzaneen, South Africa), 3) Invader-b-Lok™ (River BioScience (Pty) Ltd, Addo, South Africa) and 4) STATIC spinosad ME™ (Dow AgroSciences Southern African (Pty) Ltd.). B.I. Toolkit is a wood-fibre block that contains ME (8.2 g/block), mercaptothion EC500/UL1130 (5.0 ml/block) and a controlled release agent (3.8 g/block). Last Call is a paste-like matrix that contains ME (100 g/kg) and the insecticide permethrin (60 g/kg). Invader-b-Lok is a wood-fibre block impregnated with ME (15 g/block) and mercaptothion EC500/UL1130 (5 ml/block). STATIC spinosad ME is a gel like matrix that contains spinosad (Naturalyte) (20 g/L) and ME (510 g/L). The four male annihilation treatments were applied at the recommended rates on the product labels. Twelve fibre blocks per hectare of the B.I. Toolkit were evenly distributed on the perimeter of the mango orchards. Last Call B.I. was applied with the Last Call applicator to leave measured droplets on the leaves. Droplets were evenly distributed throughout the orchard and 150 g per hectare was applied. Invader-b-Lok was applied at 12 blocks per hectare and evenly distributed on the perimeter of the orchard. STATIC spinosad ME was applied at 300 ml per hectare as 60 droplets of 5 ml deposited on the trunks on the perimeter of the orchard. The male annihilation treatments were applied 6 weeks before harvest, and each treatment was replicated four times in 1 hectare blocks. The MAT treatments were applied for a period of 10 weeks. The tree spacing in the orchards was 7 m between rows and the different blocks were separated by at least 7 rows. In all treatments, M3 fruit fly bait stations were placed at 400 units per ha. The M3 fruit fly bait station contained a mixture of protein hydrolysate and plant extract at 5.0 g per bait station and alpha-cypermethrin at 0.01 g

per bait station. The bait stations were used together with the MAT due to the presence of other economic important fruit fly species.

Yellow Lynfield traps baited with methyl eugenol (Invader-Lure, River BioScience (Pty) Ltd, Addo, South Africa) and Chempac bucket traps baited with 3-component Biolure (Chempac Pty Ltd, Paarl, South Africa) were used for monitoring *B. dorsalis* within the treated blocks. One of each of the two traps was placed per 1 hectare block. Traps were placed out two weeks before application and serviced for a 12-week period. Additionally, four Chempac yellow bucket traps baited with methyl eugenol (Invader-Lure) were placed on the perimeter of the farm in mango orchards to monitor *B. dorsalis* at approximately 300 m away from the MAT treated areas (Fig. 1).



**Fig. 1.** Mean number (+ SE) of *Bactrocera dorsalis* males trapped with methyl eugenol baited traps in mango orchards on the perimeter of the study farm (outside of treated blocks).

The first *B. dorsalis* adults were trapped in the orchards during early January 2014. The mean number of *B. dorsalis*/trap/day in the Lynfield traps with Invader-Lure for the four different treatments was subjected to an analysis of variance. The mean number of flies present 2 weeks before application of MAT, 1 to 6 and 7 to 10 weeks after MAT application was analysed separately for each treatment. The number of *B. dorsalis*/trap/day in Chempac bucket traps with 3-component Biolure was analysed separately for each treatment.

There were no differences in the mean number of *B. dorsalis* males/trap/day in Lynfield traps with Invader-Lure between the MAT treatments (Table 1). Likewise, there were no differences in the mean number of *B. dorsalis*/trap/day in Chempac yellow bucket traps with 3-component Biolure between the different MAT treatments (Table 1). In all treated blocks, the number of *B. dorsalis* males in methyl eugenol baited traps remained below 1 male per

trap per day during treatment (Table 1) whilst outside of the treated blocks, catches went up to 1 male per trap per day indicating that M3 fruit fly bait stations and MAT were effective in suppressing population of *B. dorsalis* (Fig.1). One hundred fruit from each treated hectare were harvested at the mature green stage. Harvested fruit were transported to the laboratory and left at room temperature to ripen. Ripe fruit were evaluated for the presence of fruit fly larvae. No infested fruit were found in any of the treatment blocks, thus indicating that the four MAT treatments in combination with M3 fruit fly bait stations were equally effective in preventing infestation.

**Table 1.** Mean number of *Bactrocera dorsalis*/trap/day (with standard deviation) in blocks treated with different MAT treatments.

Trap and Lure	Treatment	Mean number of <i>B. dorsalis</i> males/trap/day		
		2 weeks before MAT	1 to 6 weeks after MAT	7 to 10 weeks after MAT
Lynfield and methyl eugenol (Invader-Lure)	M3 + Bi Toolkit	0	0.024(0.041)	0.018(0.018)
	M3 + Last Call Bi	0	0	0.089 (0.059)
	M3 + Invader b-lok	0	0.006(0.010)	0.027(0.030)
	M3 + Static Spinosad ME	0	0	0.036(0.025)
	F-value		1.00	1.93
	Degrees of freedom		3, 12	3, 12
	Probability level		0.436	0.195
Chempac bucket and 3-component Biolure	M3 + Bi Toolkit	0	0	0.018(0.031)
	M3 + Last Call Bi	0	0	0
	M3 + Invader b-lok	0	0	0.045(0.059)
	M3 + Static Spinosad ME	0	0	0.036(0.025)
	F-value			0.96
	Degrees of freedom			3, 129
	Probability level			0.454

In March 2014, the same male annihilation treatments evaluated in the mango orchards in the farm at Constantia, Limpopo Province, were re-evaluated in Star Ruby grapefruit orchards within the same farm for a period of 14 weeks. This included a pre and a post treatment week, with treatments applied on 12 March 2014. Treatments remained exposed within treated blocks for a period of 12 weeks. All treatments included a bait application treatment (M3 bait station at 240 units per ha). Each treatment was applied to a block of about 1 ha of Star Ruby

grapefruit orchard. Treatments including bait stations were removed on 4 June 2014. Six treatments were compared:

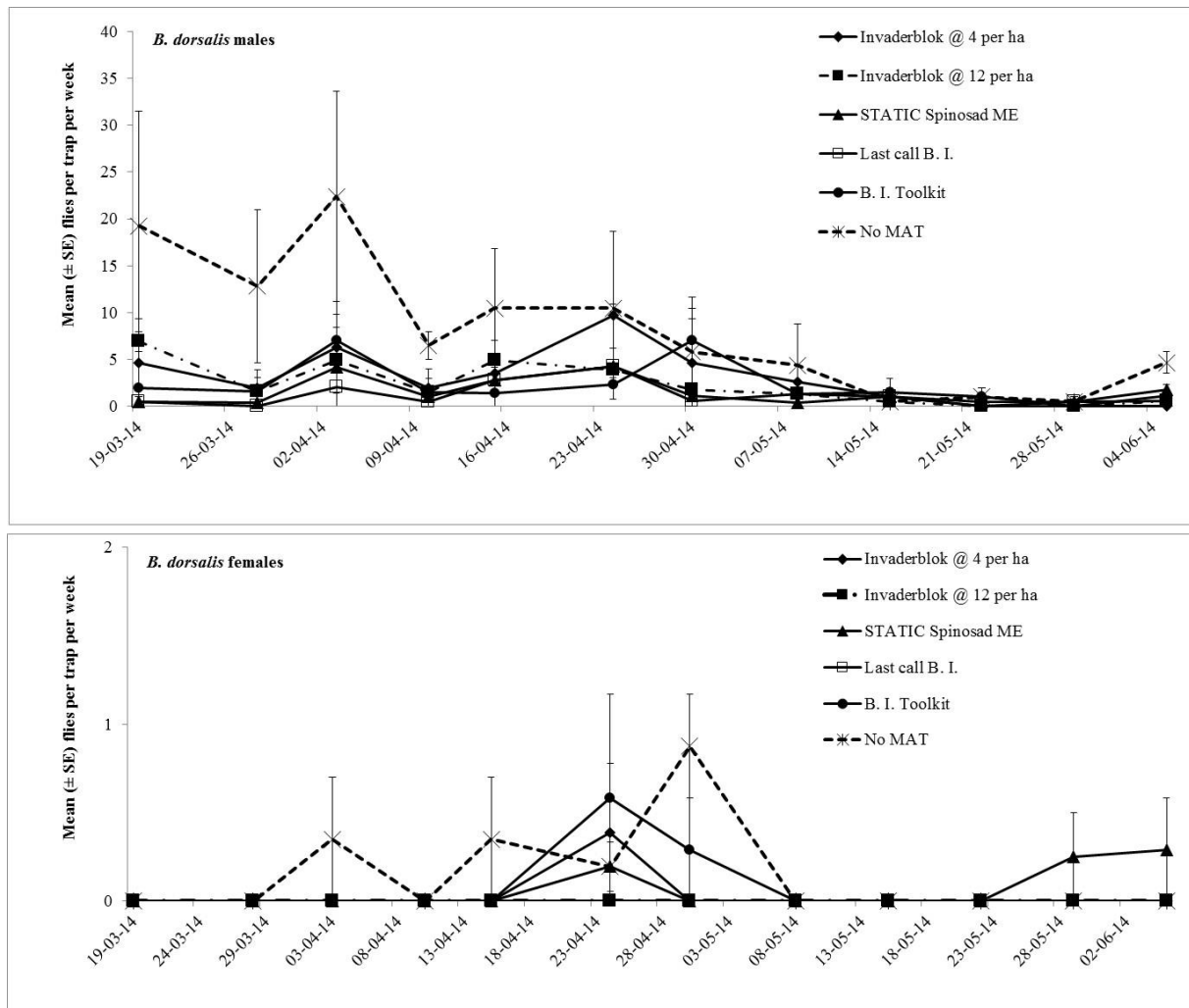
- (1) Invader-b-Lok at 4 units per ha equally distributed.
- (2) Invader-b-Lok at 12 units per ha equally distributed.
- (3) Static spinosad ME applied at 300 ml per ha, with spot applications every 49 m
- (4) Last Call B.I at 150 g/ha.
- (5) B.I Toolkit at 12 units per ha equally distributed.
- (6) No MAT (only M3 bait stations as control).

There were 2 replicate blocks per treatment in each orchard. In each block, one Lynfield trap baited with methyl eugenol (Invader-Lure), 2 Chempac bucket traps baited with Torula Yeast (ISCA Technologies, Inc., California, U.S.A) and 2 Chempac bucket traps baited with 3-component Biolure were used for monitoring of *B. dorsalis*. A fruit damage assessment was carried out at harvest where 500 fruit in each block was selected at random on the trees and visually examined for fruit fly stings. Fruit suspected to be damaged were brought to the lab, weighed and reared individually in aerated containers to confirm infestation.

Prior to treatment application, *B. dorsalis* male numbers per trap per week in blocks treated with MAT (all treatments pooled) plus M3 bait stations and those treated with only M3 bait stations averaged at  $62.72 \pm 13.57$  (SE) and  $107.48 \pm 59.65$  (SE), respectively. During treatment application, *B. dorsalis* male numbers per trap per week in blocks treated with MAT plus M3 bait stations and those treated with only M3 bait stations averaged at  $1.92 \pm 0.23$  (SE), and  $8.24 \pm 1.89$  (SE).

Results are presented in Fig.2. No females were found in any Torula Yeast baited traps and in any 3-component Biolure baited traps prior to treatment application. *Bactrocera dorsalis* females were however captured during treatment application, closer to harvest. *Bactrocera dorsalis* females were generally in higher numbers in Torula Yeast baited traps compared to Biolure baited traps. During treatment application, catches of *B. dorsalis* females per Torula yeast baited trap per week were  $0.03 \pm 0.01$  (SE) in blocks treated with MAT plus M3 bait stations, while in blocks treated with only M3 bait stations catches of *B. dorsalis* females averaged at  $0.15 \pm 0.07$  (SE) per trap per week.





**Fig. 2.** Variation in catches (mean number of flies per trap per week  $\pm$  SE) of *B. dorsalis* males (upper graphic) and females (lower graph) during the study period (March-June 2014) in Star-Ruby grapefruit blocks treated with different male annihilation treatments in combination with M3 bait stations and in blocks treated with only M3 bait stations (no MAT- No Male Annihilation Treatment). The catches presented were during the period when treatments were applied.

Although numerically, *B. dorsalis* male and female numbers were higher in blocks treated with just a bait treatment than in blocks treated with a combination of MAT and a bait treatment, there were no significant statistical differences in numbers of *B. dorsalis* males and females trapped between the different treatments (Repeated Measures ANOVA: Males:  $F_{5,121}=1.078$ ,  $P=0.456$ ; Females:  $F_{5,121}=0.820$ ,  $P=0.577$ ) (Fig. 2). No fruit fly infestation was recorded in any of the treated blocks.

#### *Evaluation of SPLAT spinosad ME as a stand-alone method*

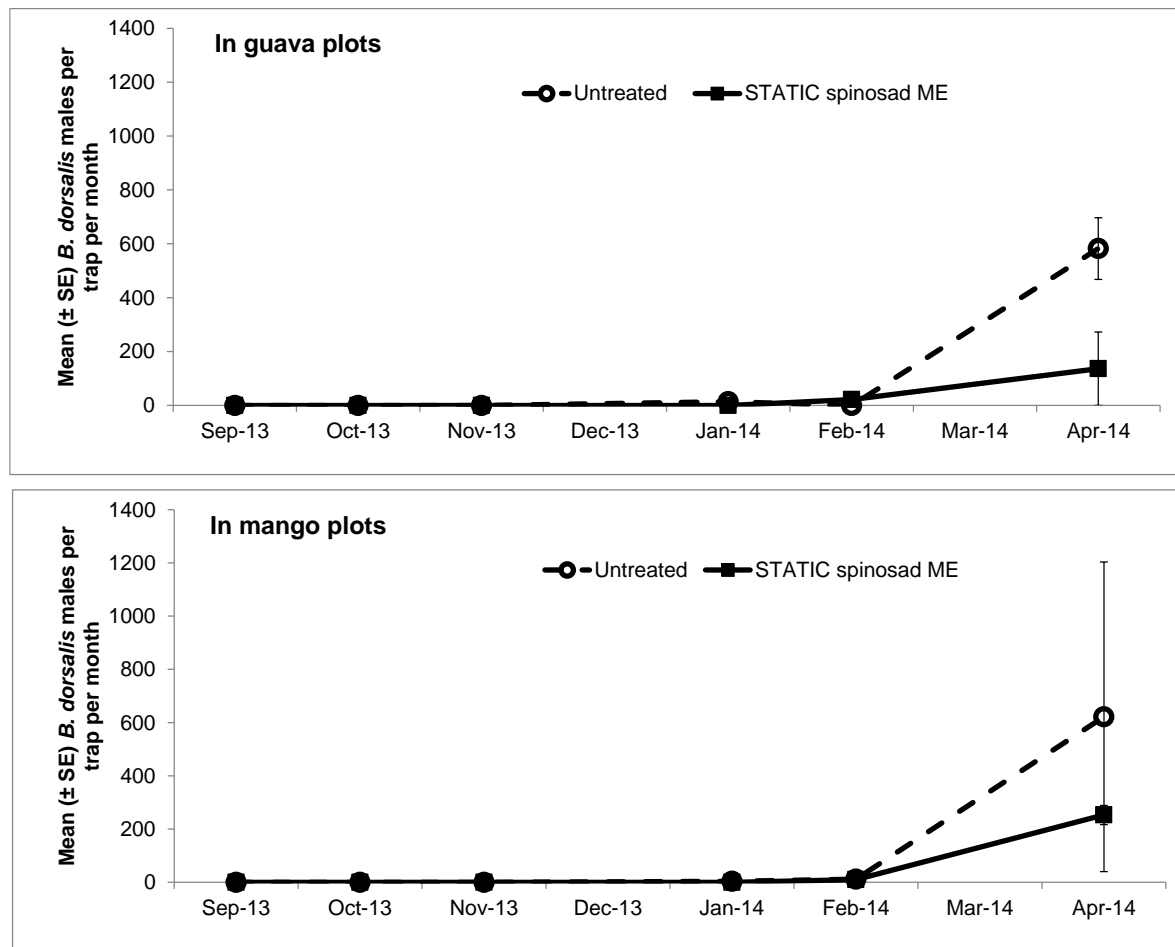
A mango orchard and a guava orchard were selected in a *B. dorsalis* infested area, in Levubu, Limpopo Province, South Africa. Both orchards were approximately 1 ha each. STATIC spinosad ME was applied in each orchard starting in the third week in September 2013 and re-

applied after approximately every two months until April 2014. Application of STATIC spinosad ME was carried out using a knapsack equipped with an adjustable applicator. The applicator was set at 20 ml, and droplets were deposited at 5 m intervals or every second tree on the edges of each orchard on wild shrubs or windbreaks. As there were no stakes or poles within the orchard, trunks of trees at the perimeters of the orchards were treated to comply with product label recommendations. Two ME baited traps were placed in each orchard where STATIC spinosad ME was applied. Additionally, two ME baited traps were placed in untreated mango orchards at 1.5 km and 4 km away from each of the orchards where STATIC spinosad ME was applied to serve as untreated areas. ME baited traps placed in the treated and untreated areas were serviced on a monthly to six weekly intervals until the end of April 2014.

Catches of *B. dorsalis* males were numerically lower in the guava orchard treated with STATIC spinosad ME compared to untreated areas near the guava orchard (Fig. 3). In the mango orchards, there were no differences in catches of *B. dorsalis* males between areas treated with STATIC spinosad ME and untreated areas (Fig. 3). In the mango orchards, there was a high variability in catches between the traps in the untreated area and this could explain the lack of differences in *B. dorsalis* male catches between the area treated with STATIC spinosad ME and the untreated area. Generally though in this trial, differences in *B. dorsalis* catches between treated and untreated areas were not statistically significant and there was also no significant effect of orchard type in catches of *B. dorsalis* males (Repeated Measures ANOVA: Treatment:  $F_{1,45}=0.899$ ,  $P=0.348$ ; Orchard:  $F_{1,45}=0.211$ ,  $P=0.648$ ).

### **Future perspectives, knowledge gaps and future research on the use of MAT on mainland Africa**

The initial results on the field studies on MAT carried out in South Africa are suggesting that a combination of MAT and bait application can reduce adult *B. dorsalis* catches in commercial orchards and can prevent fruit infestation. These initial results also do not show major differences in control efficacy between the different types of male annihilation treatments, despite that these treatments varied in the amount of ME applied per ha. However, the sample sizes of treatments evaluated in these studies were small and pest levels were low even outside the treated blocks. As such, further tests and more replications are required in order to better quantify the efficacy of MAT in control programmes against *B. dorsalis*.



**Fig. 3.** Mean number ( $\pm$  SE) of *Bactrocera dorsalis* trapped with methyl eugenol baited traps in mango and guava orchards treated with STATIC Spinosad ME and in neighbouring untreated areas in Levubu, Limpopo Province, South Africa, between September 2013 and April 2014. The first treatment of STATIC spinosad ME was in the third week of September 2013. STATIC spinosad ME was reapplied after approximately every two months until April 2014.

The cost and availability of male annihilation treatments might be the stumbling blocks in the adoption of MAT in control programmes against *B. dorsalis* across Africa. The male lure ME and ME-based products have to be imported from outside of Africa. Fibre board MAT blocks fall in the cheaper range of male annihilation products costing approximately US \$0.90 per unit and US \$10 per ha when applied at 10 units per ha (recommended rates of fibre-board MAT blocks in South Africa are between 10 and 16 per ha). STATIC spinosad ME falls in the more expensive range of male annihilation products with an estimated US \$60 per ha if 300 ml are used per ha. Since male annihilation products have to be reapplied every 8 weeks, this will amount to between US \$20 and US \$120 per ha per season (assuming a 4-month season). Small scale and subsistence farmers may not be able to carry these extra costs by themselves given that they additionally have to incur the costs of bait sprays or bait stations for control of *B. dorsalis* to be more effective. Since ME is a phenylpropanoid occurring naturally in many plant species (Tan and Nishida 2012), local natural sources of ME for MAT could be sought

in Africa in order to reduce reliance on import of synthetic ME, especially for small scale subsistence farms. Costs of MAT may be more affordable for large scale farms. The use of MAT would be imperative for protection of export crops where there is a zero tolerance of fruit fly larvae in fresh fruit produce. In large farms, products which are easier to apply and require less labour for application might be preferred.

Different types of male annihilation treatments would be suitable for different environments. Male annihilation treatments in the form of a gel-like or paste-like matrix such as STATIC spinosad ME and Last Call B.i. may be more effectively used in residential areas where placement of fibre board MAT blocks could be more difficult. MAT blocks containing the organophosphate malathion might also be less suitable in residential areas due to higher health and environmental risks associated with organophosphates. As such, for suppression and eradication programmes in Africa, it will be important to have different types of male annihilation treatments registered for the control of *B. dorsalis*.

Suppression programmes against *B. dorsalis* are likely to be more effective if they are carried out over larger areas. The use of multiple suppression techniques in combination with MAT will benefit the establishment of a systems approach for fruit fly control in particular if carried out over a large area. The expansion of an area under control ensures a better cost benefit ratio as the cost of control per ha decreases with more users on a bigger scale and it ensures better control of marginal areas, home gardens and neglected orchards (Vargas et al. 2010). Small-scale subsistence farmers would possibly not be able to carry the cost of expensive area-wide control programmes and would need subsidies from government (Barnes and Venter 2008). Commercial farmers on the other hand may be able to carry the costs only on their own land and would need support from government to cover marginal areas, home gardens and neglected production areas.

In order to improve the cost effectiveness of MAT, deployment and density of male annihilation products would have to be optimised. There are still a number of questions that needs to be answered. Is application of a MAT product more effective when applied uniformly throughout an area or would it be as effective if applied only around the perimeter of an area? How many MAT carriers are required per ha for effective control? Application of MAT methods and timing of application should also be optimised in order to reduce selection for non-responsiveness of *B. dorsalis* to ME. Results from previous studies have shown that non-responsiveness of *B. dorsalis* males could be increased by selection (Cunningham 1989b, Shelly 1997). Removal of responsive *B. dorsalis* males from a pest population could possibly promote the growth of a non-responsive strain of the pest, which could then reduce the efficacy of MAT in the long run in an area. With increasing use of MAT on the African continent, the effect of ME-based male annihilation products on non-target insects would require investigation. Non-target species, including a few beneficial insects (e.g., flower-associated insects and lacewings), have been previously reported to be attracted to ME (Suda and Cunningham 1970, Asquith and Kido 1994, Kido et al. 1996, Leblanc et al. 2009). During the surveillance programme for *B. dorsalis* in South Africa, some non-targeted fruit fly

species, such as *Perilampus* sp. and *Ceratitis millicentae* De Meyer and Copeland as well as other insects, including beneficial insects such as lacewings, were recorded in ME-baited traps. Impacts on non-target insects are important, especially when marginal and wild areas are included in area-wide suppression programmes.

## Conclusion

Given the proven efficacy of MAT for control of *B. dorsalis* in other parts of the world, it is important to further evaluate the efficacy of MAT against this pest in Africa, so it could be included in suppression and eradication programmes across the continent. Registration of male annihilation products and ME should be fast-tracked in those African countries where ME as well as ME based products are not yet registered. With regards to applied research on MAT on mainland Africa, the focus should be on optimizing the application in order to make MAT more cost effective. Application methods that would have minimal impact on the environment should also be determined.

## Acknowledgments

We would like to acknowledge technical support from J-H. Daneel, R. Beck and S. B. B. Thobela (Citrus Research International- CRI). We are grateful to Dr S. D. Moore and Dr T. G. Grout (CRI) for comments and suggestions in the previous version. We thank Insect Science (Pty) Ltd., Dow AgroSciences Southern Africa (Pty) Ltd. and RiverBioscience (Pty) Ltd. for materials provided in evaluation of MAT. This review and research were supported by Citrus Research International, Agricultural Research Council and Department of Agriculture, Forestry and Fisheries.

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# **Chemical Ecology & Attractants**

## Bait manufactured from beer yeast waste and its use for fruit fly management

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### Abstract

**Background:** A bait manufactured from beer yeast (*Saccharomyces sp.*) waste obtained from breweries has been widely used to control several species of pest fruit flies belonging to the genus *Bactrocera* (Diptera: Tephritidae) in smallholder and larger commercial fruit and vegetable farms in Asia. A two-step process involving heating to remove the alcohol and excess water, followed by enzyme treatment to fully autolyze the yeast cells is carried out in stainless steel vats. Food-grade preservative is then added to the processed beer yeast waste to prevent spoilage and provide a shelf-life of up to 2 years. The processed bait contains about 12 -18 per cent crude protein, 18 - 25 per cent sugars, and a fruity aroma.

**Methods:** Both laboratory and field trials have shown the bait to be equally or more attractive to several species of adult pest fruit flies compared to other commercially available protein baits. Commercial production of the bait has been established through an initial and older two-tank system at two locations in Vietnam, and subsequently through an improved and more efficient one-tank system with plants in Malaysia. For use in the field, 1 litre of bait is diluted in 9 litres of water and to this mixture, either malathion 0.2% a.i., fipronil 0.01% a.i or spinosad insecticide is added as the toxicant. This bait/insecticide mixture is applied weekly as a foliar low volume spot spray requiring only 10 litres per hectare, starting from fruit set until harvest.

**Results:** The bait, in conjunction with the low volume application technique, has provided impressive and consistent control of a number of species of fruit flies. In smallholder peach orchards in northern Vietnam, infestation by two species of fruit flies, *Bactrocera dorsalis* and *B. pyrifoliae*, was reduced from near 100 % to less than 4 % within one season and the industry revived. In the Mekong delta in South Vietnam infestation in Barbados cherry orchards (*Malpighia emarginata*) by *B. dorsalis* and *B. correcta* was reduced from over 70% to less than 4 %. The bait is now widely used by sapota, guava and dragon fruit farmers as well. When the insecticide-bait is used in conjunction with the male annihilation technique (methyl eugenol blocks), the resultant damage to fruits is even lower. In Bangalore, India, the bait has been used in conjunction with cue-lure blocking and crop hygiene to reduce damage in gherkin (*Cucumis sativus*) to less than 0.3 % as required by the export market. The bait is currently being trialled in area-wide fruit control programs for *B. dorsalis* (= *B. papayae*) and *B. carambolae* infesting mango in Indonesia, and for *B. dorsalis* and *B. correcta* infesting dragon fruit (*Hylocereus undatus* and *H. polyrhizus*) in Vietnam.

**Conclusions:** In many countries in Asia, the availability of locally produced baits mixed with insecticide and in conjunction with a low volume application technique, has been a major development in the region for effective management of fruit fly pests. In addition, the low volume spot spray application technique has minimal impacts on beneficial and non-target organisms and is generally safe to applicators. Importantly, the technique has provided a safe and viable alternative to the traditional and less desirable method of using cover sprays of insecticides for fruit fly control.

**Keywords:** area-wide management, *Bactrocera* species, beer yeast waste, protein bait.

## Introduction

Farming communities and fruit and vegetable industries across the Asian region struggle to cope with infestation by fruit flies (Diptera: Tephritidae), with damage ranging from 40 to near 100 per cent across a wide range of fruit and fruit vegetable crops. Several species of fruit flies are responsible for these losses with the major ones being *Bactrocera dorsalis* (Hendel), *B. carambolae* Drew & Hancock, *B. correcta* (Bezzi), *B. cucurbitae* (Coquillett), *B. latifrons* (Hendel), *B. pyrifoliae* (Drew & Hancock), *B. zonata* (Saunders) and *Dacus ciliatus* (Loew). It is to be noted that the species previously described by Drew and Hancock (1994) as *B. papayae* and *B. philippinensis* in Southeast Asia (as well as *B. invadens* in Africa) have now been synonymised with *B. dorsalis* (Schutze et al., 2015). The fruit fly problem is also exacerbated with farmers having to cope with high populations of adult flies in their farms, often all year round.

The main option open to farmers in the Asian region has been to apply cover sprays of insecticides to protect their crops. Unfortunately, this practice by smallholder farmers results in several detrimental side effects such as high pesticide residues in harvested produce, indiscriminate killing of non-target and beneficial organisms such as pollinators, parasitoids and predators, and toxicity to farmers and their families who often do not use adequate protective equipment when applying pesticides. What these farming communities desperately need is fruit fly control technology that is simple to apply, safe to users and the environment, consistently effective and reliable, and low in cost.

An effective method of controlling fruit flies is to attract adult *Bactrocera* flies in the field to a food bait that is mixed with some insecticide and applied to the crop foliage. Both sugar and protein are attractive, because following eclosion from the pupa, adult fruit flies belonging to the genus *Bactrocera* are sexually immature and have to feed on protein for the development of their ovaries and testes, as well as daily on sugar and water for their survival (Vijaysegaran et al., 2002). Adult fruit flies, particularly when they are sexually immature, are attracted and killed when feeding on the protein + insecticide mixture, thereby significantly reducing populations of adult flies in the field. Steiner (1952) first demonstrated the usefulness of this concept when he utilized hydrolysed vegetable proteins laced with insecticide (malathion) to control *B. dorsalis* in Hawaii. Several commercial formulations of hydrolysed proteins have

now been in use for fruit fly control for a number of years. Another useful source of vegetable proteins is autolysed yeast and in Queensland, Australia, a protein bait made from autolysed yeast has been used in the eradication of *B. papayae* (now *B. dorsalis*) from Queensland in the 1990s (Cantrell et al., 2002). However, these commercial protein baits are too expensive for farmers in many countries in Southeast Asia to import and use.

To address this issue and assist smallholder farmers in Asia, Vijaysegaran (1989) first developed the idea of utilizing yeast obtained locally as a waste from beer brewing to produce low cost bait in Malaysia. The first and original beer yeast waste based bait was produced and released for sale by the Malaysian Agricultural Research & Development Institute in 1989, under the commercial name of PROMAR. The bait essentially consisted of waste brewery yeast that had been autolysed by heat treatment at the brewery. He also demonstrated that applying the bait as a low volume 50 ml spot to each tree in a fruit orchard at weekly intervals from fruit set to harvest, provided more than 95% control of *Bactrocera* fruit flies infesting carambola within a single growing season of 60 days (Vijaysegaran, 1989). The total spray volume applied per week was only 10 litres per hectare and there was no need to apply the bait as a high volume cover spray (400 – 500 L/ha) as was the recommendation for ground application of protein baits to fruit crops at that time. PROMAR was widely used by carambola growers but unfortunately did not contain a preservative to extend storage of the bait under field conditions. This, along with a number of other reasons, did not enable the bait to be commercially successful and production of PROMAR ceased in the mid 1990's.

Subsequently, Lloyd and Drew (1997) picked up on this idea of using beer yeast waste to produce fruit fly bait and developed a process for treating waste brewer's yeast with heat and enzyme and adding a preservative to enable a product that had a shelf life of 2 years. Over the period 2002 – 2008, extensive work was done in Vietnam through research projects funded by the Australian Centre for International Agricultural Research (ACIAR). This work led to the construction of two beer yeast waste bait plants located within local breweries, one in Tien Giang province in the Mekong delta in the south, and the other in Hanoi in the north. Both plants are based on an older two-tank design. Together with extensive staff and farmer training, the bait spot spray technology has been widely adopted in Vietnam and has provided smallholder farmers with a low cost, effective and safe method of controlling fruit flies. The bait has since been commercialised and is sold to farmers under the trade name SOFRI Protein 10DD in South Vietnam and EntoPro in North Vietnam.

Based on the success of the beer yeast waste bait in Vietnam, in 2004 a commercial partner, Pupuk Alam Ltd in Malaysia, then constructed and commissioned an improved and more efficient one-tank system based in a local brewery in Kuala Lumpur, Malaysia. The bait is sold under the commercial name PRIMA Fruit Fly Bait. Together with the simple low volume spot spray technique, this control technology has been widely tested across smallholder and larger commercial fruit and fruit vegetable farms in Asia and has provided excellent fruit fly control. The two different bait production systems in Malaysia and in Vietnam and the results of the several control trails in the field are reported in this paper.

## Materials and Methods

### *Conversion of beer yeast waste to fruit fly bait*

Beer is produced commercially by mixing water, a source of starch which is usually malted or germinating grain, and adding yeast (*Saccharomyces* species) in large stainless steel vats to carry out fermentation. Hops may be added for flavour. At the end of the brewing cycle, beer is formed with the yeast settling at the bottom of the brewing tank. The yeast is usually reused for brewing for another 2 – 3 cycles and after this it is discarded and called spent yeast. At this stage, the spent yeast is extracted out at the bottom of the brewing tank as a slurry containing dead and live yeast cells, sugars, alcohol, and some beer and water. This slurry is also full of fermentation gases, and it froths and bubbles readily as the yeast cells are still actively fermenting. The quantity of spent yeast slurry is large and presents a waste disposal problem for many commercial breweries. The spent yeast slurry is rich in nutrients (protein, sugars, vitamins and minerals) and can be dried over steam heated drums to produce yeast flakes and sold as a supplement to formulate animal feed. However, heat drying represents a significant cost and alternative uptake and use of the spent yeast slurry is of interest to many commercial breweries.

This spent yeast waste slurry can be converted to fruit fly bait in a series of steps as follows:

#### **Raw yeast waste from brewery**

Obtain spent yeast slurry from the brewery. Use only slurry with a specific gravity of 1.03 or more.



#### **Degassing**

Place slurry in a stainless steel vat and do not fill to more than three-quarters full. Heat gently (not exceeding 40° C) while stirring continuously to remove excess gas in the slurry.



#### **Removal of excess water and alcohol**

Once the excess gas has evaporated (very little froth on the surface), increase the heat to 95°C and while stirring continuously. Keep heating at 95°C until the volume of the slurry is reduced to about half. Do not reduce volume any further. This process can take anywhere from 24 – 48 hours depending on the size of the vessel. It is very important to prevent the solution from overheating and becoming burnt at the bottom and sides of the container.



**Enzyme aided proteolysis**

Allow the solution to cool to 65°C and then add 0.2% concentration papain enzyme and heat for another 24 hours.



**Addition of preservative**

Stop heating and allow the solution to cool to 35°C. Add 0.2% food grade potassium sorbate and stir thoroughly.



**Bottling**

Cool to ambient temperature and bait is ready for bottling and use in the field.

*Commercial bait production systems*

Using the above process, bait production plants have been constructed and are in operation in Vietnam and in Malaysia. An older design two-tank system exists at two locations in Vietnam (Tien Giang province in the south, and Hanoi in the north) and an improved single tank plant is located in Shah Alam, Selangor, Malaysia.

Two-tank system

In this system, two similar sized stainless steel tanks placed next to each other are required. Heating is achieved with steam that is circulated in a jacket surrounding each tank. A boiler to generate steam is thus required. An electric motor driven agitator with Teflon scraper blades is used to continuously stir the solution and prevent the yeast solution charring at the tank bottom and sides. Degassing and evaporation of excess water and alcohol is carried out in the first tank. After this process has been completed, the reduced volume slurry is pumped into the second tank, where enzyme (papain) aided proteolysis is conducted. Many of the operations in the two-tank system are manually operated and the process flow has to be manually monitored. A single production cycle requires about 7 days to complete, depending on ambient temperatures that influence the rate of cooling.

Single-tank system

In the improved single-tank system, only one tank is required, and in which both the first stage of heating to evaporate excess water and alcohol, as well as the second stage of enzyme aided proteolysis are carried out within a single tank. The use of steam for heating and chilled water for cooling allows for more efficient processing times. The whole process flow is also fully automated and logged electronically to monitor and manage quality control. The added advantage of a single-tank system is that, if production needs to be increased, a second or



more tanks can be added on in a modular fashion to step up production volume. A single production cycle takes about 5 – 7 days to complete.

#### *Bait application in the field*

Bait produced by the above process contains about 18 – 25 % sugars, 12 – 18 % protein, water and fruity fermentation odours. For use in the field for fruit fly control, the following protocol is used:

- Dilute 1 litre of bait in 9 litres of water. Do this in a container or directly in a knapsack sprayer. Prepare the required volume of bait spray needed for use in the field using the 1:9 ratio of bait:water.
- Add one of these recommended insecticides: malathion, fipronil or spinosad to this mixture. Choice of insecticide will depend on what is available and permitted locally. Different insecticide manufacturers produce different formulations and concentrations of the insecticides. Any one of these may be used but ensure that the final insecticide concentration in the spray solution does not exceed 0.2% active ingredient (a.i) for malathion and 0.01% a.i for fipronil. For spinosad, follow the manufacturer's recommendation as stated on the label for use of this insecticide in bait sprays.
- Using a knapsack sprayer, apply a 20 - 50 ml spot of the insecticide-bait mixture to the foliage of each and every tree in the orchard, so as to apply 10 - 20 litres of spray solution per hectare of crop. A higher volume is not required. For fruit vegetable crops such as gherkins (*Cucumis sativa* L.), bitter melon (*Momordica charantia* L.), etc. that are usually grown on trellises, apply the bait in alternate rows but keep to the volume of 10 – 20 litres spray solution per hectare of crop.
- Apply the bait between 7 – 9 am and before the weather gets too hot.
- For each crop, begin bait application after fruit set and repeat applications at weekly intervals until harvest. The total number of applications will depend on the crop type.

#### *Staff and farmer training*

Training for both extension and technical staff and farmers is an integral component to ensure the success of the bait spot spray technique, and has to be planned and conducted properly. Training for technical staff and more than 5,000 farmers has been carried out in Vietnam through Farmer Field Schools comprising about 25 farmers in each batch. In the Asian Fruit Fly IPM project, farmer training is conducted through an agro-ecosystems analysis approach in farmer field schools (Kumar et al., 2011a; 2011b).

### Case studies

Over the period 2003 – 2014, together with the low volume spot spray technique, the bait has been tested across smallholders as well as larger commercially oriented fruit and fruit vegetable farming communities in Asia. Five different case studies are presented below.

#### 1) Peach cultivation by H'Mong minority in North Vietnam

The H'Mong people are an Asian ethnic minority group who traditionally live in the mountainous regions of North Vietnam (as well as China, Laos and Thailand). In order to provide an alternative to the traditional cultivation of opium poppy, the government introduced peach, *Prunus persica* (L.), and plum, *Prunus* spp., in the early 1990's. The trees adapted well to the local environment and provide a single peach crop every year with flowering in February/March and harvest in June/July. Unfortunately, two species of fruit flies, viz. *B. dorsalis* and *B. pyrifoliae*, infest the peach fruit when it starts to ripen in early June and with damage rapidly rising to near 100 per cent within 3 weeks, as the fruit ripens towards harvest in late June. Farmers were thus forced to harvest fruits at an immature green stage and many were at the verge of abandoning peach cultivation.

Trials were thus conducted over the period 2002 – 2006 in a 60 ha area in Loong Luong village, Moc Chau district in North Vietnam, comprising an entire H'mong village where peach was cultivated. First, the nature of damage by fruit flies to peach was monitored by sampling 100 peach fruits beginning soon after fruit set until harvest at 6 different stages of maturity as determined by the following colours on fruit: green (immature), light yellow, yellow, yellow-pink, pink and pink-red (fully ripe). Fruits were held individually in rearing containers for adult fly emergence and to calculate the per cent damaged by fruit flies.

For the control operations, farmers and their families were taught the standard spot spray bait protocol and the entire village was coordinated to begin bait spray applications after fruit set and to continue bait application at weekly intervals until harvest. Fruit fly bait was obtained from the bait plant in Tien Giang province, South Vietnam, and the insecticide fipronil was used at a concentration of 0.01% a.i in the bait spray solution. A separate area of 8 ha of peach located about 2 km away was used as untreated control to monitor and compare levels of fruit fly damage. Fly damage to fruit was monitored by sampling 100 peach fruits at the 6 different colour stages i.e. green (immature), light yellow, yellow, yellow-pink, pink and pink-red (fully ripe), and holding the fruit individually in rearing containers for adult fly emergence. The percentage of fly infested fruits in the control and treatment sites at the different fruit maturity stages was then calculated. Three sets of trials were carried out in 2004, 2005 and 2006. Statistical analysis of the data was conducted using Repeated Measures ANOVA (GenStat 64-bit Release 16.1) Data were also collected on the fruit yield at harvest, and incomes of farmers before and after the bait spray program was introduced.

#### 2) Barbados cherry for export in the Mekong Delta, South Vietnam

Barbados cherry (*Malpighia emarginata* DC), also known as Acerola or West Indian Cherry, is a unique fruit that is grown extensively by smallholder farmers in Go Cong province in the

Mekong Delta in South Vietnam. The plant is non-seasonal and fruits throughout the year. The fruit is sold primarily to a joint venture company in Go Cong Province that processes and exports the fruit as frozen puree to Japan. The local Barbados cherry industry provides the main source of income for several hundred families. However, two species of fruit flies, viz. *B. dorsalis* and *B. correcta*, cause extensive damage to the fruits, reducing yields and farmer incomes.

Two trials were conducted over the period 2006 – 2007 to control *B. dorsalis* and *B. correcta* using the spot spray bait technology. An area covering 30 ha for the trial conducted in 2006, and expanded to 150 ha in 2007, of Barbados cherry was chosen as the treatment area. Another 10 ha of the crop located about 2 km away where standard farmer practice (insecticide cover sprays) was used as the control site in both trials. Farmers were taught the spot bait spray technology and bait application was coordinated such that weekly bait applications were carried out over the Barbados cherry area on the same day each week.

Five hundred Barbados cherry fruits were sampled beginning 1 week before the first application of bait sprays, and then maintained at weekly intervals for 4 weeks after bait spraying was initiated. Fruits were held individually in small rearing cups and observed for adult fly emergence to determine the percentage of fruits infested in both the treatment and control sites. The first trial was carried out in 2006 and the experiment was repeated in 2007. Statistical analysis of the data was conducted using Repeated Measures ANOVA (GenStat 64-bit Release 16.1). The initial assessment or pre-bait spray period damage assessment data were not included in the analysis which was carried out for the bait spray periods of Week 2 – 5 only. Data were also collected on the fruit yield at harvest, and incomes of farmers before and after the bait spray program was introduced.

### 3) Gherkin for export in Karnataka state, India

Gherkin (*Cucumis sativa* L.) is a cucurbit crop that is extensively cultivated by over 10,000 smallholder farmers in the states of Karnataka, Andra Pradesh and Tamil Nadu in south India, through a contract farming system with a major local company called Global Green with its headquarters in the city of Bangalore. The total cultivated area is about 8,000 ha and provides a total yield of about 100 tons per day. The fruits are harvested at various sizes and pickled and bottled for export to Europe, USA, Russia and other global markets. Infestation by two species of fruit flies, viz. the melon fly *B. cucurbitae* and *Dacus ciliatus*, however, poses a very serious problem for the industry.

The critical problem for the export-based gherkin industry is that there is zero consumer tolerance to the presence of fruit fly larvae in jars of pickled gherkin. The detection of even a single fruit fly larva in a jar by consumers, particularly in Europe and USA, can lead to complaints, lawsuits and rejection of subsequent shipments. The gherkin industry is thus faced with the critical task of ensuring fruit fly larvae free gherkin through effective management of fruit fly infestation in the gherkin production sites. Control measures undertaken by farmers consist of intensive application of a variety of cover sprays of

insecticides. Despite such applications, fruit damage experienced was more than 4 %, a level which is unacceptable for processing by the factory and for export. Such insecticide cover spraying also presented the problem of unacceptable residue levels that frequently breached the maximum residue limits (MRL) acceptable to markets within the European Union, leading to additional problems for the gherkin industry in India.

To provide a solution to this problem, an area-wide management (AWM) program was carried out in an area of about 3 km<sup>2</sup> in Kashapura village, Chickaballapura district, south Karnataka, over the period December 2007 to August 2010. The AWM area consisted of a Core Zone surrounded by a 50 m wide Buffer Zone surrounding the Core Zone on all sides. Gherkin plantings consisted 10%, other crops 50% and wild vegetation and fallow area 40%, of the AWM area. The gherkin plantings ranged from 0.1 – 0.2 ha in size and each gherkin plot was surrounded by a border row of three rows of maize (*Zea mays* L.). The border row provided a roosting site for adult melon flies. The control methods applied were male annihilation (MAT), beer yeast waste bait application (BAT) and sugar baits and field sanitation. Male annihilation was carried out with 5 x 5 cm fibreboard blocks impregnated with a mixture of cue lure and chlorpyrifos, and placed in a grid at 50 m interval throughout the Core Zone and at 25 m intervals in the Buffer Zone. The cue lure blocks were replaced monthly. Beer yeast waste bait produced in Malaysia (PRIMA Fruit Fly Bait) plus 0.2% malathion was applied as a spot spray at weekly intervals to the gherkin crop. In addition, a local sugar (10 % jaggery) plus malathion spray was applied to the maize grown as a border crop surrounding each plot of gherkin. Adult melon fly populations were monitored at weekly intervals using cue lure baited traps placed in the AWM area. Fruit fly damage to gherkin fruits at harvest was determined by examination of fruits which had been harvested daily and brought to the processing factory. Fruits were examined for oviposition punctures and dissected for presence of larvae. Such damage assessments were carried out over three seasons with several thousand fruits being examined in the factory. Gherkin production areas about 2 km away and outside the AWM zone were used as a control site.

#### 4) Area-wide management of fruit flies in smallholder mango farms in Indonesia

Mango (*Mangifera indica* L.) is an important cash crop and is cultivated in smallholdings that extend over large areas in the provinces of Indramayu, Cirebon and Majalengka in the island of Java, Indonesia. Production, however, is affected by two species of fruit flies, *B. dorsalis* (previously *B. papayae*) and *B. carambolae*. An area-wide fruit fly management program (AWM) was started in 2011 using a combination of male annihilation, bait spraying, crop hygiene and farmer training. The AWM program was implemented in two sites totalling 100 ha in size (De Faveri et al., 2014).

The two trial sites were located in Krasak (40 ha) and Sliyeg Lor villages (60 ha) in the district of Indramayu, province of West Java. A standard insecticide cover spray area of mango was used as a control site at Jambak Village (40 ha). The combined control methods were used simultaneously at the treated sites. For male annihilation, 5 x 5 cm fibreboard blocks soaked in a 4: 1 methyl eugenol and fipronil insecticide mixture were used all year

round and continuously throughout the trial. The blocks contained about 12 ml of the mixture and were nailed onto trees at 50 m intervals (approx. 6 blocks/ha), within the orchard as well as in the village. Blocks were renewed every 2 months. Bait sprays using PRIMA Fruit Fly bait manufactured from brewery yeast waste in Malaysia were applied weekly in the orchards beginning at fruit set and ending at harvest with malathion 0.2% a.i. used as the toxicant. Four spots of 25 ml each were applied per tree (100 ml/tree). This resulted in a volume of about 10 litres of spray mixture/ha/application. Crop hygiene included the removal and destruction of fallen and unwanted mango fruit as well as removal of major alternative fruit fly hosts around dwellings in the village. Adult fruit fly populations were monitored at weekly intervals with the use of methyl eugenol + fipronil baited Steiner-type design traps. Damage to mango fruits at harvest was assessed at the packing house. Fruit was visually observed and suspected damaged fruit was incubated individually in containers to confirm the presence or absence of larvae and to estimate fruit infestation levels.

Populations of adult fruit flies surrounding and outside the AWM zone in Krasak were also monitored, using a separate group of 8 methyl eugenol baited traps placed along the four cardinal points at a radius of 1 and 2 km from the centre of the AWM zone. Traps were serviced weekly, captured flies counted and flies per trap per day (FTD) values were calculated.

#### 5) Area-wide suppression of *Bactrocera* fruit flies in dragon fruit in Vietnam

Dragon fruit (*Hylocereus undatus* (Britton & Rose) and *H. polyrhizus* (Britton & Rose)) is a major export-oriented crop in Vietnam and is grown largely in Binh Thuan province. Two species of fruit flies, *B. dorsalis* and *B. correcta*, infest dragon fruit and pose a constant threat to the export oriented industry. The current control practice of insecticide cover spraying on a farm-by-farm basis has been found to be inefficient, polluting and is becoming increasingly unacceptable to foreign markets. A pilot area-wide fruit fly control program was thus initiated in 2013 to (1) suppress fruit fly populations over large areas of dragon fruit and (2) to assess the economic benefits that can be achieved through the implementation of an area-wide IPM program (Khanh et al., 2016).

The AWM area consisted of a 100 ha core zone (1 km x 1 km), surrounded on all sides by a 300 ha buffer zone (2 km x 2 km). The dragon fruit area outside the buffer zone consisted of existing farmer practices and was used as a control site. Starting in July 2013, treatments applied in the Core Zone were (1) sanitation, consisting of stripping and destruction of unwanted dragon fruit and removal of alternate hosts where possible; (2) male annihilation (MAT) using methyl eugenol + fipronil fibreboard blocks placed at 50 m intervals, and; (3) a mixture of beer yeast waste bait (EntoPro 150 DD) mixed with fipronil 0.01% a.i. and applied weekly as a spot spray to the foliage of alternate host trees (50 mL/tree) and on the dragon fruit plants at a rate of 10 litres/ha of bait + insecticide mixture during the dry season (mid-April to mid-June). In the rainy season (May to October), a mixture of 3 parts EntoPro in 7 parts water + 1g of fipronil 800WG was placed in bottle traps (200 mL mixture/bottle) and distributed in the dragon fruit orchards at a density of 25 traps per ha. In the buffer zone, only

sanitation and MAT were applied as described above. In the farmer practice or control zone, some farmers used one methyl eugenol baited trap on every third dragon fruit plant in their orchards, some applied insecticide cover sprays occasionally, and other farms did not apply any control methods at all. Adult fruit fly populations in all three zones were monitored continuously with methyl eugenol + fipronil baited Steiner-type design traps. Traps were serviced weekly, captured flies counted and FTD values were calculated.

To assess fruit fly damage (*B. dorsalis* and *B. correcta* combined) in the core, buffer and farmer practice zones, ripe dragon fruits were collected at random and the fruits were held individually over sterilized sawdust in rearing containers and monitored for adult fly emergence. In the core zone, 160 fruits were sampled each month, while in the buffer and farmer practice zones, 480 fruits were sampled each month. The fruits were scored as either infested (1 or more pupae or adults emerging) or uninfested (no pupae or adults emerging) and the percentage infestation was calculated.

In addition, 7 other alternate fruit fly hosts found growing in the trial area were sampled for fruit fly infestation. The fruits sampled were rambutan (*Nephelium lappaceum* L.), rose apple (*Syzygium malaccensis* L.), custard apple (*Annona squamosa* L.), guava (*Psidium guajava* L.), Barbados cherry (*Malpighia emarginata* DC), jujube (*Zizyphus mauritiana* Lam.), and mango (*Mangifera indica* L.).

## Results

### Case Studies

#### 1) Peach cultivation by H'Mong minority in North Vietnam

The typical infestation pattern of peach fruit by *B. dorsalis* and *B. pyrifoliae*, as shown in Table 1, reveals that infestation by fruit flies increases very rapidly within a very short window of time of just 1 week when the fruits are changing in colour from light yellow (5% infested) to yellow (67% infested). Thereafter, fly damage kept increasing until it reached 100% in the sample of pink-red (ripe) fruit. This infestation trend is important because any control measures have to be applied and be very effective before heavy damage to peach sets in within this short window of time.

**Table 1.** Infestation pattern of *Bactrocera dorsalis* and *B. correcta* in peach in Moc Chau in 2002.

Sampling date	Fruit colour/maturity	Weight of 100 fruits (kg)	% infested out of 100 fruits sampled
26 May 2002	Green (immature)	3.56	0
1 June	Light yellow	4.08	5
8 June	Yellow	4.13	67
16 June	Yellow-Pink (half ripe)	4.17	81
23 June	Pink	5.37	93
30 June	Pink-Red (fully ripe)	5.50	100

The results of the application of bait sprays in a coordinated program over 60 ha of peach conducted over 2004 - 2006 are shown in Table 2.

**Table 2.** Infestation levels of *B. dorsalis* and *B. pyrifoliae* combined in bait sprayed peach orchards (60 ha) compared to unsprayed orchards (8 ha control) in Loong Luong Village, Moc Chau District, North Vietnam over a three year period from 2004-2006.

Fruit colour/maturity	% fruits infested out of 100 fruits sampled at each maturity stage							
	Year 2004		Year 2005		Year 2006		Average over the 3 year period*	
	Control	Bait Spray	Control	Bait Spray	Control	Bait Spray	Control	Bait Spray
Green (immature)	0	0	0	0	7	0	2.3a	0.0a
Light yellow	3	0	0	0	10	0	4.3a	0.0a
Yellow	58	3	6	0	45	0	36.3b	1.0a
Yellow-pink (half ripe)	90	5	41	3	91	1	74.0c	3.0a
Pink	95	4	79	2	100	3	91.3cd	3.0a
Pink-red (fully ripe)	100	5	100	4	100	4	100.0d	4.3a

\*Means followed by the same letter within a column or row are not significantly different (LSD mean separation test,  $P = 0.05$ ).

Throughout the three years (2004, 2005 and 2006) the bait spray studies were conducted, a typical and consistent pattern of fruit fly infestation was observed in both unsprayed and sprayed peach orchards, with fly damage being nil or very low in green immature fruit. In the unsprayed orchards, however, damage then increased rapidly when fruits were about half ripe and rose to 100% in the samples of fully ripe fruit. There was no difference in damage in the control site calculated over 3 years between green and light yellow fruit. However, damage increased rapidly in yellow fruit and was higher than in less mature fruits. Damage increased as fruit maturity increased from half ripe to fully ripe fruits. In the bait spray areas, this increased infestation with fruit maturity was not observed, and infestation was lower in sprayed orchards than in unsprayed orchards for all maturity stages from half ripe to fully ripe.

The drastic reduction of fruit fly infestation in the 60 ha bait spray area resulted in an increase in yield and quality of peach fruit harvested. The H'Mong villagers had never before experienced this since the crop was established. Essentially, fruits could be left on the trees and harvested when they were larger and ripe instead of being harvested small and green. Harvesting of ripe fruit has led an increase in productivity and incomes and the data are provided in Table 3.

**Table 3.** Productivity and income from bait sprayed compared with untreated peach orchards in Loong Luong Village, Moc Chau District, North Vietnam in 2006.

Concept	Untreated peach orchards	Bait spray peach orchards
Average yield per ha (kg)	6,000	10,000
Price per kg of fruit (Vietnamese Dong - VND)	1,000 VND	4,000 VND
Income/ ha	6,000,000 VND (USD \$ 400)	40,000,000 VND (USD \$ 2,666)

The application of beer yeast waste bait sprays resulted in a six-fold increase in incomes within a single growing season. And very importantly, as a result of these increased incomes, the H'Mong villagers have stopped cutting down their peach trees and have returned to profitable peach growing.

## 2) Barbados cherry for export in the Mekong Delta, South Vietnam

The results of the application of bait sprays in a coordinated program over 150 ha of Barbados cherry conducted in 2006 and 2007 are shown in Table 4. Before the application of bait sprays, fruit fly damage was fairly similar with an average over 2006 and 2007 at 58% and 51.5 % in the control and treatment plots respectively. With the introduction of bait sprays, fruit fly damage decreased rapidly to a low of 4% in the fourth week after starting bait spraying compared with 59.5% in the insecticide treated area. Fruit fly damage over the 4 week period bait sprays were applied (weeks 2 to 5) and over the 2 years the trials were conducted (2006 and 2007) was lower in the bait spray areas than in the insecticide cover spray areas (Table 4).

**Table 4.** Fruit fly infestation levels in bait sprayed compared with insecticide cover sprayed Barbados cherry orchards in Go Cong District, South Vietnam in 2006 and 2007.

Date	% infested out of 500 fruits sampled each week					
	2006		2007		Average*	
	Insecticide cover spray (10 ha)	Bait spray (30 ha)	Insecticide cover spray (10 ha)	Bait spray (150 ha)	Insecticide cover spray areas (10 ha)	Bait spray areas (30 ha)
Before bait spray (week 1)	67	50	49	53	58	51.5
1 <sup>st</sup> spray (week 2)	58	30	53	42	55.5	36.0
2 <sup>nd</sup> spray (week 3)	62	15	36	23	49.0	19.0
3 <sup>rd</sup> spray (week 4)	60	10	57	7	58.5	8.5
4 <sup>th</sup> spray (week 5)	47	4	72	4	59.5	4.0
<b>Average (week 2 to week 5)</b>	<b>56.8</b>	<b>14.8</b>	<b>54.5</b>	<b>19.0</b>	<b>55.6a</b>	<b>16.9b</b>

\* Values with a similar letter are not significantly different (LSD mean separation test,  $P = 0.05$ ).



The significant reduction in fruit fly damage resulted in improved yields and better quality fruits at harvest, leading to higher prices and increased incomes. Data collected in 2007 on productivity and incomes are shown in Table 5.

**Table 5.** Productivity and income from bait sprayed compared with untreated Barbados cherry orchards in Go Cong District, South Vietnam in 2007.

	Untreated orchards (insecticide cover spray)	Bait spray orchards
Average yield per ha (tons)	20	25
Price per kg of fruit (Vietnamese Dong - VND)	1,500 VND	3,500 VND
Income/ ha	30,000,000 VND (USD \$ 1,875)	87,500,000 VND (USD \$ 5,470)

The use of bait sprays has led to increased yields, prices and subsequently close to a 3-fold increase in incomes for Barbados cherry farmers.

### 3) Gherkin for export in Karnataka state, India

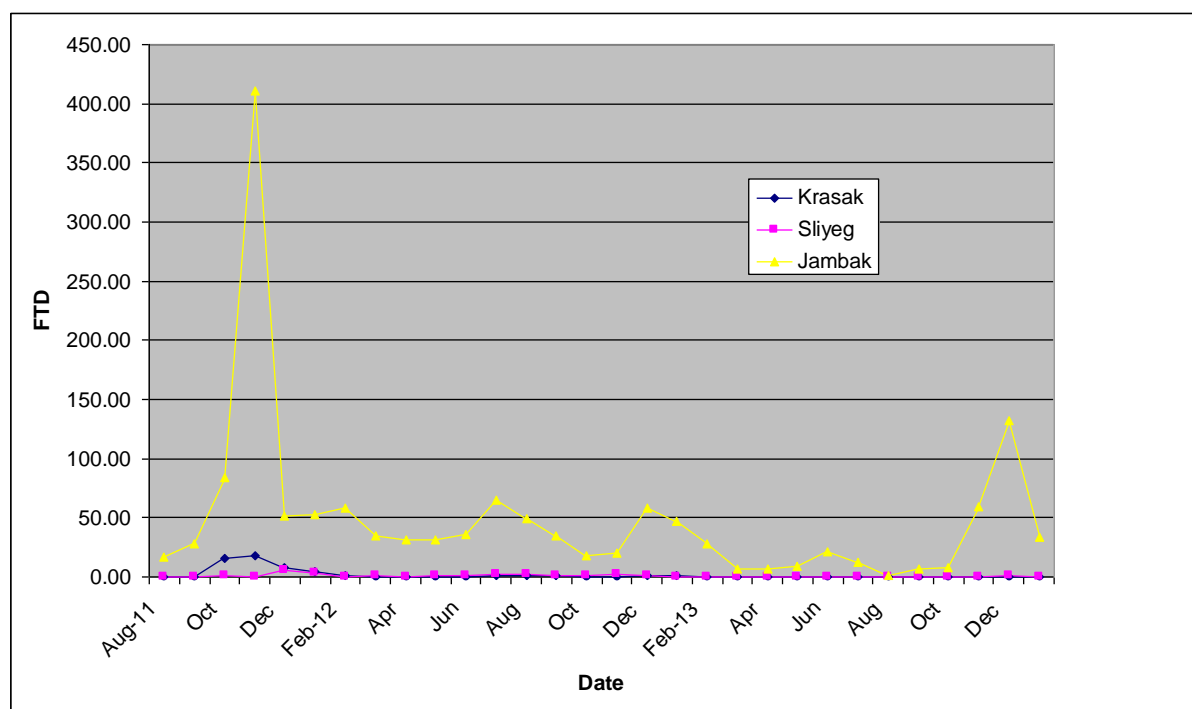
Before the initiation of the AWM program in December 2007, the FTD was 1.21. After December 2007 with the implementation of the AWM program, the FTD fell to 0.07 during the 4<sup>th</sup> week of 2008. Thereafter, FTD was mostly less than 0.5 and never exceeded a value of 0.84. In the gherkin production area outside the AWM zone, however, FTD values frequently exceeded 1, and with a high of 2.37 in 2008, 3.55 in 2009, 2.83 in 2010 (Praveen et al., 2012).

With regards to fruit fly infestation, damage to harvested gherkin in the AWM area was generally less than 0.7 % and with a peak of 1.42 % experienced once in 2009. In comparison, fruit damage outside the AWM area ranged from a low of 3.66 % to a high of 5.64 % over the trial period (Praveen et al., 2012). Subsequent continuation of the AWM suppression program has led to fruit fly infestation of less than 0.3 %, and has enabled the gherkin industry in southern industry to meet with the stringent quality requirements and sustain export markets (Munikote and Mouli, 2014).

### 4) Area-wide management of fruit flies in smallholder mango farms in Indonesia

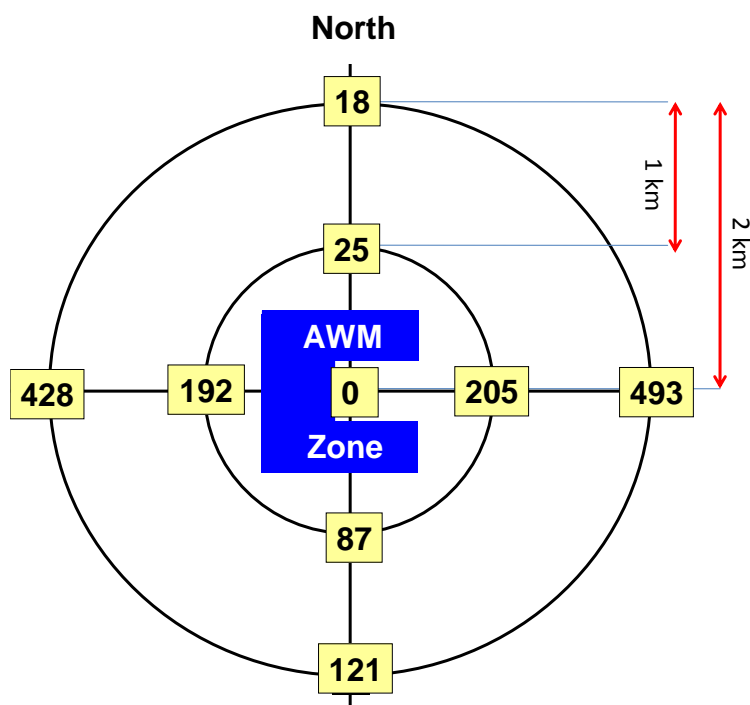
The effectiveness of the AWM suppression program as indicated by FTD for each monthly period starting in August 2011 to January 2014 is shown in Fig.1. The FTD values indicate that fly populations were very high and fluctuated widely in conventionally treated mango farms at Jambak (60 ha) but were very effectively suppressed in both the AWM zones at Krasak (40 ha) and Sliyeg Lor (40 ha). In the 2012 mango season, the combined treatments in the AWM zones reduced fruit fly populations by 98% compared to the control or insecticide cover sprayed production area. In early 2013, the FTD was at a low of 0.22 and 0.12 in Krasak and Sliyeg Lor respectively, compared with a very high value of 20 at the control site in Jambak. Infestation by fruit flies in 2012 was 1.6% in the AWM areas of Krasak and Sliyeg

Lor and 4.7% at the control site in Jambak. Overall, the AWM program has been successful in reducing fruit fly populations to non-economic levels. Growers also claim that fruit quality has improved and they are obtaining higher prices from wholesale buyers for their higher quality fruit.



**Fig. 1.** The number of flies/trap/day (FTD) for each month starting in August 2011 to December 2013 recorded in the area-wide fruit fly suppression zones of Krasak and Sliyeg Lor, compared with the control site at the conventionally treated (insecticide cover sprayed) area in Jambak, Java, Indonesia.

The FTD values of the traps located in the area surrounding the AWM zone in Krasak at a radius of 1 and 2 km from the zone centre indicate that extremely high populations of adult fruit flies were recorded in the areas surrounding the AWM zone (Fig. 2). These results demonstrate that it is possible to create a fruit fly low prevalence zone in ecosystems and production areas where high endemic populations of fruit flies exist using a combination of bait sprays, MAT and sanitation.



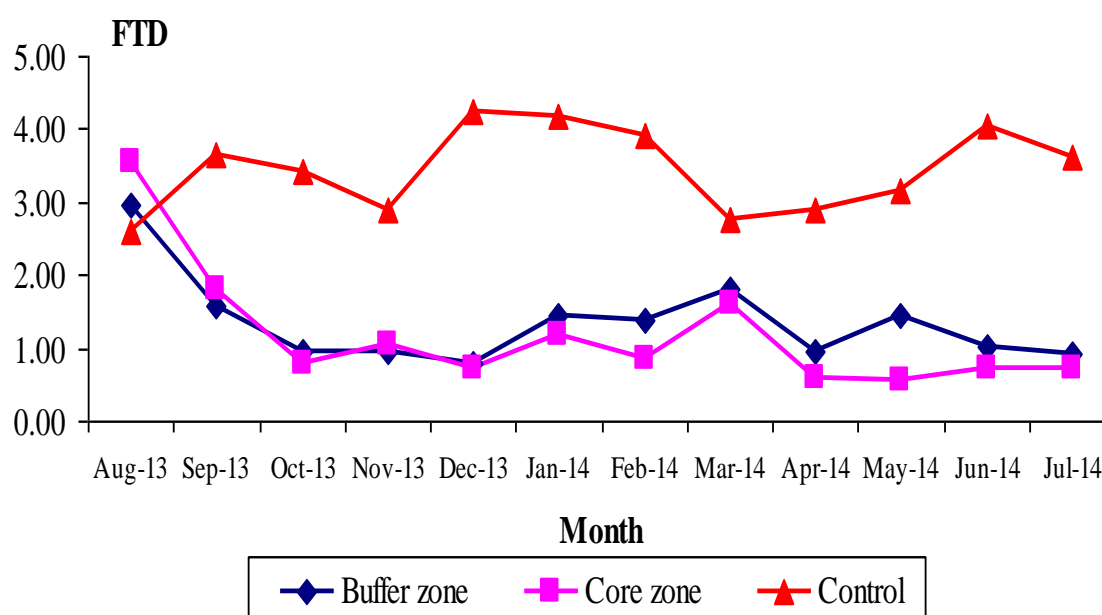
**Fig. 2.** The number of flies/trap/day recorded in methyl eugenol baited traps placed along the four cardinal points and at a radius of 2 km away from the centre of the area-wide management zone in Krasak, Java, Indonesia.

##### 5) Area-wide suppression of *Bactrocera* fruit flies in dragon fruit in Vietnam

The effectiveness of suppression of male flies in the core and buffer zones compared with the farmer practice zone is evident from the FTD trend over a period of one year from the start of the AWM program in July 2013 until July 2014 as shown in Fig. 3 (Khanh et al., 2016, in press).

In the core zone, FTD decreased from a high of 3.5 at the start of the AWM program in August 2013 to a low of 0.7 in July 2014. The FTD in the buffer showed a similar trend and decreased from 3.0 to 0.9 over the same period. In contrast, the FTD in the farmer practice zone was consistently higher, ranging between 2.6 and 3.6. Importantly, this trial further demonstrates that the FTD over a large area of cultivation can be effectively lowered to and maintained at a value of less than 1 using a combination of male annihilation, bait spraying and orchard sanitation.

Corresponding with the reduction in FTD values, there was also a reduction in fruit fly infestation in dragon fruit in both the main and secondary seasons as a result of the AWM program (Table 6).



**Fig. 3.** The number of flies/trap/day (FTD) for each month starting in August 2013 to July 2014 recorded in the area-wide fruit fly suppression program conducted on dragon fruit in Binh Thuan, Vietnam (Khanh et al., 2016).

**Table 6.** The damage caused to dragon fruit by *B. dorsalis* and *B. correcta* combined in the area wide fruit fly suppression program conducted in Binh Thuan province, Vietnam (extracted and summarised from Khanh et al., 2016).

	Average % fruit fly infested dragon fruit		
	Core zone	Buffer zone	Farmer practice zone
Secondary season :			
October 2013 to February 2014	2.9	3.5	5.9
Main season :			
June to September 2014	2.5	5.7	9.9

The core zone experienced the lowest level of fruit fly damage, which was about half and one-quarter in the secondary and in the main dragon fruit seasons respectively compared to the farmer practice zone. Thus the AWM program has provided significant economic benefits to growers.

Besides dragon fruit, seven other types of fruits that were sampled all showed much lower levels of fly infestation in the core and buffer zones combined compared with the farmer practice zone (Table 7).

The consistent reduction in fruit fly damage in seven different alternate host fruits in the core and buffer zones compared with the farmer practice zone confirms the effectiveness of the area wide fruit fly suppression program on dragon fruit in Binh Thuan province, Vietnam.

**Table 7.** Comparison of the damage caused by *B. dorsalis* and *B. correcta* combined to seven different types of fruits growing within the area wide fruit fly suppression program in Binh Thuan province, Vietnam (extracted and summarised from Khanh et al., 2016).

Fruit type	% damaged fruit	
	Core + buffer zones	Farmer practice zone
Rambutan	6	20
Rose apple	15	42
Custard apple	0	8
Guava	14	56
Cherry	31	71
Jujube	19	29
Mango	18	65

## Discussion and Conclusions

Bait sprays produced from beer yeast waste, together with a low volume spot spray application technique, have provided excellent control of several different species of fruit flies in a number of fruit and vegetable crops in the Asian region. When combined with MAT, crop sanitation and alternate host removal, and carried out with extensive farmer training over large production areas in an area-wide program, highly effective fruit fly suppression and decrease in fruit damage has been achieved.

From the various trials carried out over the period 2002 – 2014, a general pattern has emerged regarding the use of bait sprays alone and in conjunction with other control technologies. These conclusions are summarised in Table 8.

**Table 8.** Summary of the efficacy beer yeast waste bait sprays alone and in conjunction with other control technologies in control programs conducted in the Asian region.

Method	% infested fruit	FTD value	IAEA Trapping Guidelines
BAT alone	4 (within 1 season)	> 1	Infested Area
BAT + MAT + sanitation	< 0.3 (over successive seasons)	1.0 – 0.1	Suppression (also leading to Low Prevalence)
BAT + MAT + Sanitation + Buffer Zone	(under evaluation)	0.1 – 0.0	Eradication (also leading to Free Area)

When bait sprays were applied alone without any other additional control measures, it was possible to reduce the fruit fly damage from 100 % to about 4 % within a single growing season. More intensive application of the bait did not reduce the damage any further. Fruit damage can be significantly reduced but the production area when surveyed will provide an FTD value of > 1, meaning it is considered as infested according to the IAEA Trapping Guidelines (2003). When BAT is used in conjunction with MAT and sanitation, the fruit damage can be reduced to less than 0.3 % and the corresponding FTD value ranges from 1 to 0.1, meaning it is considered as under effective suppression. When BAT is combined with

MAT, sanitation and alternate host removal, and conducted within a Core Zone surrounded by a Buffer Zone where only MAT and sanitation are used, the FTD value can be reduced to less than 0.1, which is a value used to evaluate if an area is under the Eradication phase.

Fruit and vegetable production in most countries in Asia is smallholder-based and takes place in an environment where very high endemic populations of fruit flies exist, both within and surrounding the traditional production areas. It is expected that such high fruit fly populations in surrounding zones would pose intense pressure on population suppression system applied within the growing zones. However, it has been demonstrated that fruit fly populations can be very effectively suppressed and maintained at very low levels within traditional fruit and vegetable production areas, even though these areas may be non-isolated and surrounded by other areas harbouring high endemic populations of fruit flies. The levels of suppression which range from FTD 1 – 0.1 (Suppression) to < 0.1 (Eradication) should encourage further work to be done to achieve areas of low pest prevalence leading to compliance with ISPM 30 (2008) – Establishment of Areas of Low Pest Prevalence for Fruit Flies (Tephritidae).

Other area-wide fruit fly management programs conducted in the Asian region have also reported effective suppression of pest fruit flies. A major program is in Thailand, where an area-wide integrated control program covering 70 km<sup>2</sup> of mango smallholdings in two provinces (Ratchburi and Pichit Province) was initiated in the year 2000 with the objective of controlling two species of fruit flies *B. dorsalis* and *B. correcta* that caused yield loss and quality degradation in mango (Orankanok et al., 2007). The control program used a combination of orchard sanitation, MAT, selective application of bait sprays (hydrolysed protein bait) and supplemented with the release of sterile flies. This integrated approach reduced damage to mango from over 80% before program implementation to an average of less than 3.6% in Ratchburi Province over a period of 5 years from 2000 – 2004. In Pichit Province, the fly infestation was reduced from 42.9% to 15.5% over the period 2003 – 2004.

The various AWM programs in the Asian region using bait produced from beer yeast waste in combination with male annihilation and orchard sanitation have also provided highly effective suppression of pest fruit flies, from low prevalence to near eradication levels (Table 9). Economic assessments and feasibility studies carried out in Thailand (Enkerlin, 2001; Knight, 2002) showed that fruit fly control using an integrated area-wide approach with an SIT component provided significant economic returns. The next step would be to incorporate the release of sterile flies to achieve even more effective and sustained suppression, and very importantly, with the benefits being shared over a large production area by smallholders and larger commercial farms alike. Currently, the majority of fruit and vegetable farms in Asia rely on control by individual farmers in their farms and such efforts are usually costly and ineffective in providing good control. The area-wide management approach has been demonstrated to be highly effective and should be promoted in the region with the addition of an SIT component considered for the future.

## Acknowledgements

I thank the organisers of the 9<sup>th</sup> ISSFEI for inviting me to share my experiences on fruit fly management with the international fruit fly community. The work reported in this paper is the result of many field trials conducted over two decades and in collaboration with numerous colleagues in Malaysia, Thailand, Vietnam, Indonesia, Cambodia, India, China and Australia, who are too many to list individually. In particular, Dr Le Duc Khanh and staff of the National Institute of Plant Protection in Hanoi, and Dr. Le Quoc Dien and staff of the Southern Fruits Research Institute in Tien Giang province, Vietnam, helped to carry out the trials on peach and Barbados cherry respectively. The data from the trials in Indonesia were presented at the 9<sup>th</sup> International Symposium on Fruit Flies of Economic Importance held in Bangkok in 2014, and are reproduced here with the permission of the senior author Stefano De Faveri (Department of Agriculture and Fisheries Queensland). The Australian Centre for International Agricultural Research (ACIAR) has also supported and funded a large amount of the control trials in Malaysia, Vietnam and Indonesia. Joanne De Faveri (Department of Agriculture and Fisheries, Queensland) assisted with statistical analysis of the data. All this assistance is gratefully acknowledged.

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## Search for new fruit fly attractants from plants: A review

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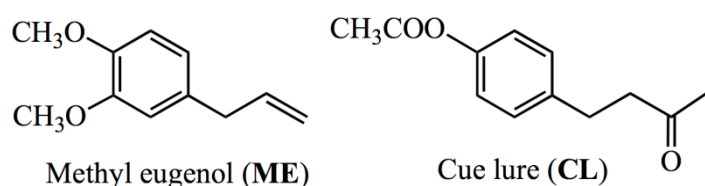
### Abstract

The fruit fly genus *Bactrocera* comprises more than 500 species including many fruit pests of economic importance. Males of many species show strong affinity either to methyl eugenol (ME) or cue-lure (CL) (or raspberry ketone (RK), its deacetyl derivative). However, there are about 200 species, which do not respond to these potent lures. We briefly review findings of new attractants, especially for ‘non-responding’ species particularly those endemic agricultural pests, through investigations of the flies’ natural interactions with plants and also by analyzing specific chemicals accumulated in the secretory organ of wild male flies. Orchid flowers of many *Bulbophyllum* species attract *Bactrocera* fruit fly males by emitting specific volatiles such as ME or RK for pollination, in the rain forests of Southeast Asia. In addition, other unknown floral volatiles in these “bactroceroophilous orchids”, strongly associated with *Bactrocera* species, are being investigated. The male flies ingested floral or plant volatiles and selectively either accumulate or biotransform the compounds in the rectal pheromone glands. A series of phenylpropanoids and phenylbutanoids, besides ME and RK, have been identified from a number of *Bulbophyllum* species. Zingerone (ZN) characterized from *Bu. patens* and *Bu. baileyi* attracts both ME-sensitive and RK-sensitive males. ZN has been subsequently found to be a specific male attractant for *B. jarvisi* in Australia. A specific attractant was isolated and characterized as 3-hydroxy- $\alpha$ -ionone from a variety of host eggplants where *B. latifrons* males congregate and compulsively feed on. Several 3-oxygenated  $\alpha$ -ionone analogs have been developed as monitoring agents for *B. latifrons* in Okinawa, Japan. A sesquiterpene hydrocarbon,  $\beta$ -caryophyllene, has been identified from the rectal glands of wild *B. correcta* males. It is apparently more attractive than ME, and thus, serves as a new monitoring agent for the species. The strong attractiveness of fruit fly males to these plant allelochemicals is a unique characteristic in *Bactrocera*. This appears to be linked to the male pheromone and defence systems within the highly diverged and diversified species radiating from central Southeast Asia.

**Keywords:** *Bactrocera*, *Bulbophyllum*, floral synomone, fruit fly attractant, orchid, phenylbutanoid, phenylpropanoid.

## Introduction

The fruit fly genus *Bactrocera* is one of the largest tephritid groups comprising more than 500 species, many of which are fruit pests of economic importance (Drew & Romig, 2013). Males of many *Bactrocera* species show strong affinity to either methyl eugenol (ME) or cue-lure (CL) (Fig.1) (or the deacetyl analog of CL, raspberry ketone (RK)) (Metcalf & Metcalf, 1992; IAEA, 2003; Tan et al., 2014). However, there are several important and key pest species, which do not respond to these lures, such as *B. cucumis*, *B. latifrons*, *B. pyrifoliae* and *B. tsuneonis* (Table 1). For the last years, we have been seeking new attractants for these ‘non-responding’ species, particularly those that are endemic agricultural pests, by observing the flies’ natural interactions with plants which have strong affinity for various *Bactrocera* species. Furthermore, we also analyze specific chemicals accumulated in the wild male flies’ secretory organs that are suspected to store volatile pheromone obtained from unknown natural sources. Here, we review our approaches for developing potential attractants, particularly for pest species not known to respond to ME or CL, based on chemoecological, biochemical and physiological analyses in the interactions between *Bactrocera* fruit flies and plants.



**Fig. 1.** Two major male attractants of *Bactrocera* fruit flies.

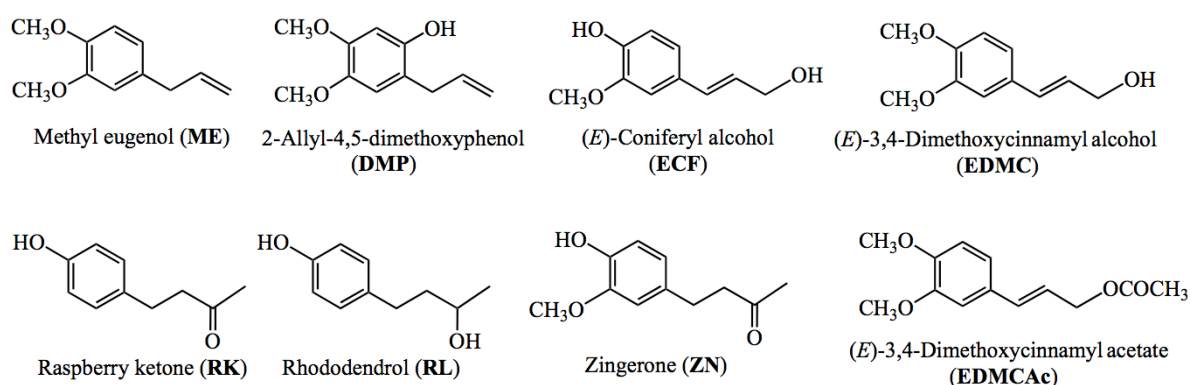
**Table 1.** Important pest species of *Bactrocera* fruit flies – responsive and non-responsive to specific male lures.

Methyl Eugenol (ME)-Sensitive	Cue-Lure (CL)-Sensitive	Non-Responsive to ME and CL
<i>B. carambolae</i> Drew & Hancock	<i>B. albistrigata</i> (Meijere)	<i>B. atrisetosa</i> (Perkins)
<i>B. correcta</i> (Bezzi)	<i>B. caudata</i> (Fabricius)	<i>B. cucumis</i> (French)
<i>B. dorsalis</i> (Hendel)*	<i>B. cucurbitae</i> (Coquillett)	<i>B. latifrons</i> (Hendel)
<i>B. musae</i> (Tryon)	<i>B. frauenfeldi</i> (Schiner)	<i>B. minax</i> (Enderlein)
<i>B. umbrosa</i> (Fabricius)	<i>B. tau</i> (Walker)	<i>B. pyrifoliae</i> Drew & Hancock
<i>B. zonata</i> (Saunders)	<i>B. tryoni</i> (Froggatt)	<i>B. tsuneonis</i> (Miyake)

\* *B. dorsalis*, *B. invadens*, *B. papayae* and *B. philippinensis* have been synonymized to a single biological species as *B. dorsalis* (see Schutze et al., 2015, and Hee et al., 2015b).

## Potential attractant from bactroceroophilous orchid flowers

Flowers of many orchid species, the so-called “bactroceroophilous orchids”, from the section *Sestochilus* of *Bulbophyllum* (Orchidaceae) selectively attract *Bactrocera* fruit fly males by emitting fragrant chemicals consisting of either ME or RK, for pollination in the rain forests of Southeast Asia and Oceania (Tan, 2009). The orchid habitats are superimposable over areas where a diversity of *Bactrocera* species inhabits. Thus, those fruit fly males act as specific pollinators for the orchids. Although the frequency was not very high, some *Bactrocera* fruit flies captured by ME and CL traps carried pollinarium on their back, suggesting pollination interactions with *Bulbophyllum* orchids in Papua New Guinea (Clarke et al., 2002). While the orchid gain reproductive benefits via cross pollination, the pollinator male flies also obtain the floral volatiles as their pheromone ingredients to attract their conspecific females (Tan & Nishida, 2000). Therefore, the floral substances involved in the specific orchid flower-fruit fly interactions are defined as “floral synomone”, a blend of semiochemicals that benefit both orchids and fruit flies. After a search for floral volatiles in these bactroceroophilous orchids that are strongly associated with *Bactrocera* species, we found a series of phenylpropanoids and phenylbutanoids besides ME and RK, from a number of *Bulbophyllum* species as described below (Fig.2). As such, plant species can be grouped according to the volatiles they produced and release.



**Fig. 2.** Phenylpropanoids and phenylbutanoids (bottom row - RK, RL & ZN) identified from floral volatiles of *Bulbophyllum* orchids.

## ME-producing orchids

Flowers of *Bulbophyllum cheiri* release ME as a major volatile; and strongly attract males of *B. dorsalis* and several other ME-sensitive species such as *B. carambolae* and *B. umbrosa* (Tan et al., 2002). The flower temporarily traps a pollinator fruit fly between its lip and column by the hinged action of the highly mobile lip structure. Besides ME, a series of related phenylpropanoids, including eugenol (EU), (Z)-methyl isoeugenol, (E)-methyl isoeugenol, 2-allyl-4,5-dimethoxyphenol (DMP), 5-allyl-1,2,4-trimethoxybenzene (eugarone), (E)-3,4-dimethoxycinnamyl acetate (EDMCAc), (E)-coniferyl alcohol (ECF) (Fig.2), were detected as

relatively minor floral volatiles (Nishida et al., 2004). DMP and ECF are known as the male sex pheromone to attract *B. dorsalis* females by emitting in the air as a smoke in the courtship sequence (Nishida & Fukami, 1990; Tan & Nishida, 1996; Hee & Tan, 1998). A mixture of DMP and ECF induces a female's acceptance posture during the process. Thus, the compounds, particularly ECF, in the flower would also attract females. However, females have never been observed visiting the flower. This is probably because the females' sensitivity to the male rectal pheromone is only limited to a short distance; and the mode of action of the molecule - as an arrestant rather than as an attractant (Nishida et al., 2000). Among the analogs, DMP alone captured as many *B. dorsalis* males as ME in the field, while ECF hardly captured flies probably due to its less volatile nature.

Flowers of *Bulbophyllum vinaceum* also emit ME as a major volatile, and effectively attract *B. dorsalis* and other ME-sensitive fruit flies. In addition, *Bu. vinaceum* flowers also produce a large array of phenylpropanoids including EU, DMP, ECF, (Z)-coniferyl alcohol (ZCF), eusarone, (E)-3,4-dimethoxycinnamyl alcohol (EDMC), and EDMCAc (Fig.2) (Tan et al., 2006). The floral architecture is unique in this orchid with the hinged lip being "spring loaded" and always kept in a "closed" position. The lip has the highest concentration of ME. Thus, an attracted fruit fly eventually climbs on to the lip and forces the lip into the open position. The fly then aligns itself along a U-shaped longitudinal channel of the lip. As it feeds along the lip, it ultimately passes a point of imbalance and it is catapulted on to the floral column. The fly quickly retreats and consequently touches the viscidium of the pollinarium and thereby, removes the pollinia stuck on to the thoracic dorsum. After removing the pollinia, the fly then disembarks from the lip which immediately springs back to a "closed" position (Tan et al., 2006). Interestingly, the floral content of ECF often doubles the quantity of ME, suggesting a short distance arrestant activity of this less volatile component together with other phenylpropanoid analogs. Yet, we still do not know the real function of those analogs accompanying the floral ME in the attraction of the fruit flies. As to whether the floral ME-analogs are to enhance the attractiveness of floral ME, or they are targeting a particular *Bactrocera* species as the most effective pollinators by emitting specific chemical blends in the natural environment or habitat, certainly warrant further investigation.

It is noteworthy to point out that there are other orchid species, namely, *Phalaenopsis violacea* (Kaiser, 1993), and *P. bellina* (Hsiao et al., 2006) that attract *B. dorsalis* males. Nevertheless, due to the immobility of the floral lip, i.e. lacks a dynamic lip mechanism to tip a fly against the floral column to either remove or deposit pollinia, the fruit flies are just floral visitors, not pollinators, that pick up floral ME or its analogs during their visits. In this case, the floral ME or its analogs may be used as chemical defense to deter insect "florivory" (McCall & Irwin, 2006) or act as anti-microbial agents.

### **RK-producing orchids**

Flowers of *Bu. apertum* (synonym - *B. ecornutum*) are scented with RK that attracts several fruit fly species, namely, *B. albistrigata*, *B. caudata*, *B. cucurbitae*, *B. melastomatos*, and *B. tau* (Fig.2). RK is mainly concentrated in the movable hinged lip, where the flies eventually

probe and feed on its surface (Tan & Nishida, 2005). A smaller amount of 4-(4-hydroxyphenyl)-2-butanol (rhododendrol) (RL) accompanies RK in most cases. Although RL appeared less attractive than RK in preliminary tests against *B. cucurbitae*, both compounds strongly stimulate feeding once the fly settle on the chemical source. RK and RL are also found in flowers of several other *Bulbophyllum* orchids such as *Bu. praetervisum* (Borneo) and *Bu. hahlianum* (Papua New Guinea) (unpublished data). RK was initially found in flowers of melon fly (*B. cucurbitae*) attracting orchid, *Dendrobium superbum* (synonym - *D. anosmum*) (Nishida et al., 1993). In this case, however, the distribution of RK is restricted to petals and *B. cucurbitae* males compulsively lick on the petal surface. The attracted males never probe on the relatively large lip. Therefore, they do not go near to the floral column; and are not pollinators of the *Dendrobium* flowers.

### **ZN-producing orchids**

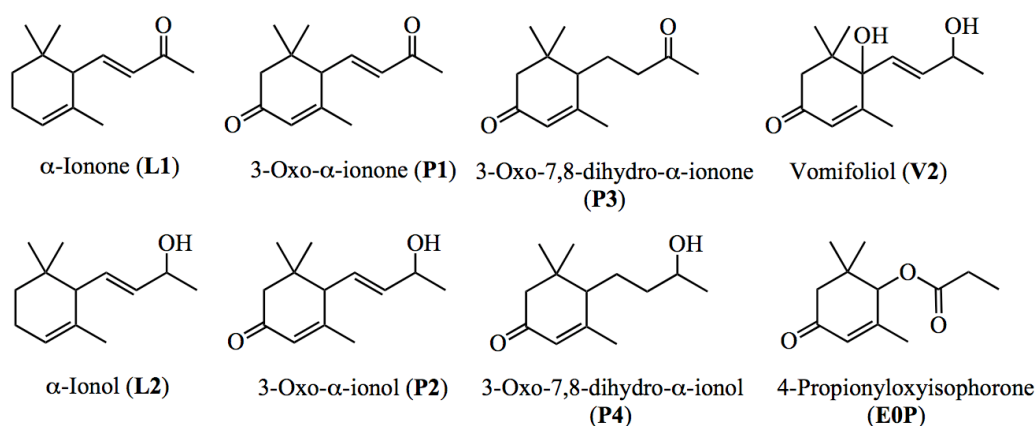
Zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-butanone) (ZN) (Fig.2), a pungent essence of ginger, has been characterized from flowers of *Bu. patens* and *Bu. baileyi* (Tan & Nishida, 2000, 2007). ZN conforms to a phenylbutanoid structure resembling both ME and RK, and this may be the reason why both ME-sensitive and RK-sensitive species (e.g. *B. dorsalis*, *B. umbrosa*, *B. cucurbitae*, and *B. caudata*) are attracted to ZN. It is likely that the flowers can secure effective pollinators in the complex rain forest habitats by attracting both ME-sensitive and RK-sensitive males. Initially, it has been observed that males of *B. jarvisi*, known as a pest of mango fruits in Australia, are strongly attracted to *Bu. baileyi* flowers (May, 1953). ZN has been subsequently found to be a specific male attractant for *B. jarvisi*, useful as a monitoring agent (Fay, 2012). Two other non-pest species in Australia *B. aglaiae*, and a new species *B. speewahensis* were also trapped using ZN as an attractant-bait (Fay, 2012). In northern Australia, *B. jarvisi* seems to serve as the most reliable pollinator species for *Bu. baileyi* flowers.

### **Attractants from other plant sources**

Besides the bactrocerophilous orchids described above, flowers, fruits, leaves or/and other parts of various plants are known to contain potent attractants for the male fruit flies, particularly for *B. dorsalis* and its related species, mainly because ME is widely present as an essential oil component in these plants (Tan & Nishida, 2012). In addition, a fragrant lei flower, *Fagraea berteriana* (Loganiaceae) in Hawaii was found to contain a series of phenylpropanoid components that strongly attract *B. dorsalis* males even though the flower entirely lacks ME. This flower contains EDMC, EDMCAc and (*E*)-3,4-dimethoxycinnamaldehyde which were characterized as the specific male attractants (Fig.2) (Nishida et al., 1997). Likewise, EDMC and EDMCAc are present in flowers of *Spathiphyllum cannaefolium* (Araceae) in Southeast Asia (Chuah et al., 1997). Although these compounds showed lower attractiveness to *B. dorsalis* males, EDMC stimulated the same level of feeding activity as ME. Since ME is suspected to be carcinogenic to mammals, alternative lure chemicals may be sought in similarly fruit fly-attracting plant sources in nature.

Among the pest fruit fly species with unknown male attractants,  $\alpha$ -ionone (L1) and  $\alpha$ -ionol (L2) (latilure) were found as specific attractants for the solanaceous fruit flies, *B. latifrons*, during the chemical screening using olfactometry (Fig.3) (Flath et al., 1994). Although the attractiveness of these norisoprenoids was weak, the lure activity was significantly enhanced by cade oil (McQuate et al., 2004, 2013). However, the potency of these attractant mixtures appeared not as high as that of ME for *B. dorsalis* or CL for *B. cucurbitae*. Some efforts were put into a survey for potential chemical lures for *B. latifrons* particularly in orchids and other flowers native in Southeast Asia by releasing male flies into a large fine-wire meshed enclosure of a butterfly farm. But observations showed none of the flies settled on or showed attraction to any of the large varieties of flowers available (unpublished data).

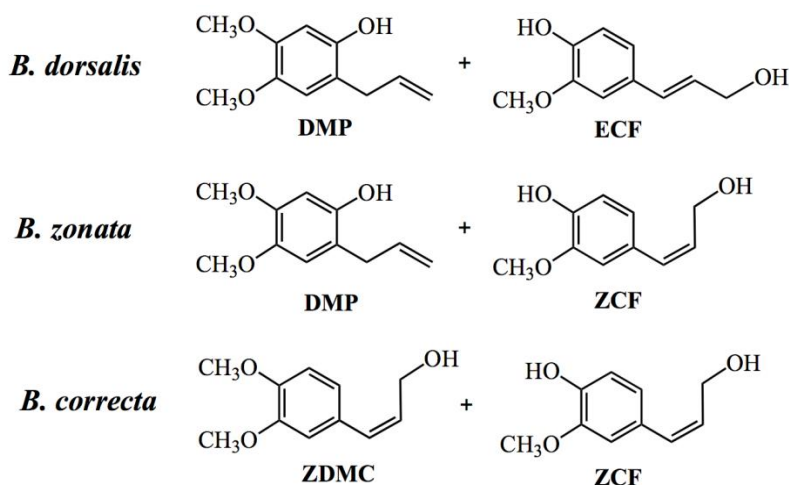
During the search for solanaceous host fruit volatiles to attract *B. latifrons* females, a variety of eggplants commonly sold in a grocery store in Hawaii was found to strongly attract males of *B. latifrons* rather than females (Nishida et al., 2009). The males voraciously feed on the eggplant tissues and its extracts, similar to the phenomenon shown by *B. dorsalis* males compulsively licking on ME-containing plants. One of the active principles was identified as 3-hydroxy- $\alpha$ -ionone (Nishida et al., 2009). Ishida et al. (2008) also found isophorone (3,5,5-trimethylcyclohex-2-enone) and isophorol (3,5,5-trimethylcyclohex-2-enol) as moderate attractant for *B. latifrons* males, which significantly enhanced activity of L1 and L2. Based on that knowledge, a series of 3-oxygenated  $\alpha$ -ionone analogs (P1, P2, P3 and P4) have been synthesized and their individual activities were evaluated (Fig.3) (Ishida et al., 2008; Enomoto et al., 2010). 7,8-Dihydro-3-oxo- $\alpha$ -ionone (P3) was shown to be highly effective (Enomoto et al., 2010). Therefore, it has been selected as a possible candidate for use as a monitoring agent for *B. latifrons* in Okinawa. Further survey also revealed vomifoliol (V2) - isolated from roots of lamb's ear, *Stachys byzantina* (Labiatae), was found to be an extremely potent phagostimulant for the male flies, although long distance attractiveness was very weak, due to its low volatility (Yoshida, Y. et al., unpublished data). Along with the above information, several analogs with an oxygen function on the C-4 position of isophorone have been synthesized to improve the attractiveness. As a result of testing, 4-propionyloxyisophorone (E0P) is being developed as a powerful lure against *B. latifrons*, particularly more effective when used in combination with P3 (Eguchi, T. et al., unpublished data). E0P is a propionate of 4-hydroxyisophorone (E0), and the ester evaporates much faster than P3 at ambient temperature, even though they possess almost similar molecular sizes.



**Fig. 3.** *Bactrocera latifrons* male attractants/phagostimulants.

### Male rectal glands as lure sources

Males of *B. dorsalis* voraciously feed on ME and biotransform it into two metabolites, DMP and ECF, in an approximate ratio of 1:1 and store them in the rectal glands (Nishida et al., 1988a). DMP and ECF serve as sex pheromone to attract conspecific females (Tan & Nishida, 1996; 1998). Likewise, upon compulsive feeding on ME, males of the peach fruit fly, *B. zonata*, accumulate DMP and (Z)-coniferyl alcohol (ZCF) in a 1:1 ratio; whereas males of the guava fruit fly, *B. correcta*, convert ME to ZCF and (Z)-3,4-dimethoxycinnamyl alcohol (ZDMC), also detected in some *B. dorsalis* males (Nishida et al., 1988b) in a 1:1 ratio (Fig.4) (Tan et al., 2011).



**Fig. 4.** Male rectal gland volatiles in 3 *Bactrocera* species after methyl eugenol consumption.

Contrastingly, males of *B. carambolae* sequester only ECF (together with endogenous volatiles) (Tan & Nishida, 1996; Wee et al., 2007). These species-specific combinations of rectal volatiles are considered to play a critical role in differentiating sympatric species if encountered in the natural habitat.



It would be ideal, if we can develop a female-attracting lure from glandular volatiles from male rectal sacs. However, a trap dispensed with the rectal components does not attract females, at least in *B. dorsalis*, probably because of the dynamic mode of rectal volatile emission as a smoke with vigorous wing-fanning action of *Bactrocera* males (Kuba & Sokei, 1988). Female's responses also may only be restricted within a short range and only during dusk (Hee & Tan, 1998). Nevertheless, DMP, a rectal component, strongly attracts males in the field, although the relative potency may not be as high as that of ME as discussed above (see Section 1) (Nishida et al., 1988a).

In the case of ME-sensitive species, males convert ME to oxygenated metabolites as mentioned above. In contrast, RK-sensitive species, such as wild males of *B. cucurbitae*, often possess large quantities of RK or ZN in the rectal gland. Since laboratory reared males lack these compounds in the gland, wild males must have acquired these substances from certain chemical sources containing RK or ZN - being attracted by such lure chemicals that are sequestered without biotransformation. Therefore, if we could analyze a rectal gland from a wild fly whose male-lure is unknown, we may be able to deduce and determine the right lure chemical(s) specific to the species.

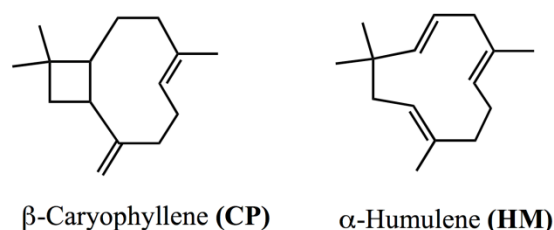
In the case of *B. latifrons*, we initially found a series 3-oxygenated- $\alpha$ -ionone/ionols (P1, P2 and P4) built up in rectal glands of males that voraciously fed on a male-attracting variety of eggplant (see section 2) (Nishida et al., 2009). Thus, the potential lure chemicals were readily determined before directly analyzing a complex mixture of eggplant fruit extracts where almost invisibly small amounts of lure chemicals were present (under an ion-extract mode in GC-MS analysis). In other words, males selectively concentrate a lure chemical by ingesting from certain plant tissues and eventually store it either intact or biotransformed as a conspicuous/major component in the rectal gland.

*Bactrocera correcta* males are attracted to ME and sequester ZDMC and ZCF in the rectal glands as mentioned above (Fig.4). Interestingly, survey of wild *B. correcta* flies in Thailand has revealed that males store extremely large quantities of sesquiterpenic hydrocarbons, such as  $\beta$ -caryophyllene (CP) and  $\alpha$ -humulene (HM) in the rectal glands in addition to or instead of ZCF and EDMC. Total amounts of sesquiterpenes often exceeded 250  $\mu$ g/gland (Fig.5) (Tokushima et al., 2010). A field test was conducted using one of the rectal components - CP, in a fruit orchard in Thailand (Fig.6). CP, a common plant constituent, has been shown to act as a highly potent male attractant for *B. correcta*. Its attractiveness appears to be higher than that of ME, whereas the co-inhabiting *B. dorsalis* was never captured by CP-traps (Chinvinijkul, S. et al., unpublished data). Additionally, *B. correcta* males respond to CP earlier than their attraction to ME nearing or at sexual maturity (unpublished data). This is highly desirable in a mass-trapping or male annihilation program to reduce mating of feral females. In a monitoring program of both *B. correcta* and *B. dorsalis*, CP can be employed as a useful measure to differentiate the trap catches of each species, or possibly as a highly selective mass trapping agent for *B. correcta*. Sequestration of these sesquiterpenes in the pheromonal gland in *B. correcta* males poses a great contrast to that in *B. dorsalis* males,

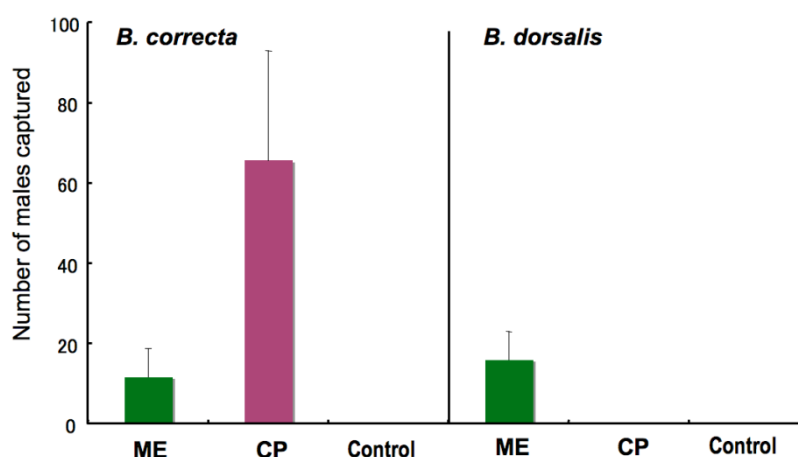
suggesting a potential role of these unique rectal ingredients in the interspecific interactions between these sympatric species.

### Concluding remarks

It was just a century ago that Howlett (1915) first discovered the strong attraction of *Bactrocera* fruit flies to ME in a citronella oil (*Cymbopogon nardus*, Poaceae), and since then, ME has been used as a lure for monitoring, surveillance and eradication programs of *B. dorsalis* and other ME-sensitive species (Koyama et al., 1984; Metcalf & Metcalf, 1992; Tan et al., 2014). Over 450 plant species from 80 families have been known to contain varying amounts of ME in the essential oils from various parts of plants, including flowers, fruits and roots (Tan & Nishida, 2012). Contrastingly, CL was discovered as a melon fly male attractant by an intensive screening program of synthetic compounds and some plant essential oils (Beroza & Green, 1963). Thus, CL was and still is regarded as a non-natural substance, whereas its deacetyl compound RK is found naturally in many plants (Tan & Nishida, 2005). Intriguingly, we have recently detected the presence of CL in one of the bactroceroophilous orchids, *Bulbophyllum hortorum*, as a minor component of the floral fragrance (Katsute, T., et al., unpublished data).



**Fig. 5.** Sesquiterpene lures specific for *Bactrocera correcta* – identified from the fly male rectal glands.



**Fig. 6.** Number of *B. correcta* and *B. dorsalis* males captured in methyl eugenol (ME) and  $\beta$ -caryophyllene (CP) baited-traps in Thailand (Chinvinijkul, S. et al., unpublished data).

As reviewed here, we have successfully found a number of natural *Bactrocera*-male specific lures by chemical analyses of (1) floral synomone in the mutually interacting orchid flowers with fruit flies, (2) other fruit fly-attracting plant sources, and (3) rectal pheromone gland volatiles sequestered from natural sources detected in wild males of certain target fruit fly pest species. Some structural modifications of such “lead compounds” were attempted to specifically design analogs with a better affinity for fruit flies. We still need to pursue new types of lure chemicals particularly for the economically important pest species, such as *B. cucumis*, *B. pyrifoliae* and *B. tsuneonis*, which do not respond to the known lures. It should also be noted that, even among the ME-sensitive species such as *B. correcta* and *B. carambolae*, males show much lower sensitivity to ME when compared with *B. dorsalis* (Wee et al., 2002; Tan et al., 2014; Hee et al. 2015a). Apparently, *B. correcta* may have shifted to using sesquiterpene hydrocarbons (CP and HM) possibly due to a female’s preference for a male pheromone composition in assessing male vigor and suitability during courtship. Although such unique pheromone systems of *Bactrocera* fruit flies have not been fully understood, the affinity to these lure chemicals appears to be strongly linked to the flies’ pheromone and defensive systems specifically developed within the highly diverged species radiating from central Southeast Asia (Tan & Nishida, 1998; Tan, 2009). It is possible that certain orchid flower may selectively attract only a specific group of *Bactrocera* species in a runaway process between the orchid and a pollinator by prescribing a specific blend of fragrance, as frequently found in pollination syndromes (Dobson, 1994). In this context, although our approach may provide new clues useful for effective pest management targeting a specific insect pest species, an extensive and intensive use of such attractants near a natural orchid habitat may threaten the mutualistic interactions between the organisms relying upon each other via specific floral synomones. Further effort is needed to fully understand the life history of these fruit flies in nature, especially in close association with both natural and agricultural ecosystems.

## Acknowledgments

This work was supported by many collaborators in various countries over 25 years. We are thankful to our colleagues and friends particularly those listed below:

- a) *Bactrocerophilous* orchids–fruit flies: A. Lamb, Y. C. Toong (deceased), A. K. W. Hee, S. L. Wee, P. T. Ong, P. O’Byrne, H. Lim and V. Yap (Malaysia), L. T. Tan (England), M. Nakahira, and T. Katsute (Japan), N. H. S. Howcroft (PNG), and J. J. Vermuelen (The Netherlands);
- b) *B. latifrons*: T. Ishida, A. Hamagami, H. Enomoto, T. Kohama, D. Haraguchi, R. Ukuda, T. Matsuyama, Y. Yoshida and T. Eguchi (Japan), T. E. Shelly and E. B. Jang (USA);
- c) *B. correcta*: W. Orankanok, and S. Chinvinijkul (Thailand), H. Ono, and I. Tokushima (Japan) and
- d) Others: S. de Faveri and H. Fay (Australia), I. Nishi, Y. Higashiura and S. Ohno (Japan).

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## Responses of *Dacini* (Tephritidae: Dacinae) fruit flies to novel male attractants in Australia and Papua New Guinea

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### Abstract

**Background:** The male lures cue-lure and methyl eugenol (ME) attract more than half of known *Dacini* fruit fly species and have been used successfully in fruit fly monitoring and control over the last 50 years. Among the fruit flies that don't respond to these lures are several pest species. Without a male lure their monitoring and control is difficult, as monitoring is limited to less attractive and messy wet protein or orange ammonia trapping or time consuming host-rearing, and control is limited to protein bait sprays or cover sprays. Zingerone, a phenylbutanoid found in orchids, was found to weakly attract both cue- and ME-responsive fruit fly species in Malaysia. Zingerone and other chemical compounds were field tested in northern Australia several years ago and found to attract several non-responsive fruit fly species. This study aimed to determine the response of dacine fruit flies to zingerone and novel eugenol analogues in Australia and Papua New Guinea.

**Methods:** Zingerone and three promising eugenol analogs from the earlier Australian trials were field tested more widely in north-east Queensland and Papua New Guinea (PNG), areas of high *Dacini* fruit fly diversity with over a 100 and 200 species respectively. Lures were applied to dental wicks with the toxicant maldison, set in Steiner traps and tested in comparison to cue-lure and ME. Catches were cleared weekly to fortnightly for 26 months in Australia and four months in PNG.

**Results:** The eugenol analogues were found to attract several 'non-responsive' species including the PNG guava pest *B. obliqua* and Australian species *B. halfordiae*, *B. barringtoniae*, *B. bidentata* and *B. murrayi*. Several cue- and ME-responsive species responded more strongly to the new lures, including the Australian cue-responsive pest *B. kraussi*, which responded most strongly to isoeugenol. Many species responded to more than one lure with varying degrees of attraction to each lure.

**Conclusions:** This study reinforces that *Dacini* male lure response is more complex than the previously held belief that fruit flies are either cue-lure or ME responsive or not responsive to male lures, and that fruit flies can have varying responses to a number of lures.

**Keywords:** *Bactrocera*, *Dacus*, male lure, trapping.



## Introduction

For decades it was generally thought that all *Bactrocera* and *Dacus* species may be categorised into three groups based on their response to either cue-lure (over 200 species) or ME (84 species) or their non-response to these lures (286 species) (Drew, 1974; Drew et al., 1982; Metcalf & Metcalf, 1992; IAEA, 2003).

Apart from the discovery of the species-specific Vertlure for *D. vertebratus* Bezzi in Africa (Hancock, 1985) and development of Latilure for *B. latifrons* (Hendel) (McGovern et al., 1989; McQuate & Peck, 2001; Enomoto et al., 2010; Nishida & Tan, 2014), fruit fly attractant research has largely focused on analogues of cue-lure or ME for invasive pests in non-endemic areas, particularly the Oriental fruit fly *B. dorsalis* (Hendel) and melon fly *B. cucurbitae* (Coquillett) in Hawaii (e.g. Beroza & Green, 1963; Mitchell et al., 1985; Metcalf et al., 1986; De Milo et al., 1994; Liquidó et al., 1998; Oliver et al., 2002; Casana-Giner et al., 2003; Khrimian et al., 2009; Jang et al., 2011). However, any lures tested where there is a limited number of invasive species will only provide information on how those particular species respond to those compounds but not on potential responses of other species.

More recently in Malaysia, zingerone was identified as the attractive compound in the flowers of the *Bulbophyllum* orchids on which fruit flies congregate (Tan & Nishida, 2000; 2007). When field tested in Malaysia zingerone was found to weakly attract several cue- and ME-responsive species. In field tests in Australia zingerone was highly attractive to *B. jarvisi* (Tryon) (Fay, 2011), a minor pest species previously recorded as only very weakly attracted to cue-lure (Drew, 1989; Royer & Hancock, 2012). Zingerone also attracted a new species (*B. speewahensis* Fay & Hancock), two ‘non-responsive’ species, *B. aglaiae* (Hardy) and *B. aurea* (May), a rare species not known previously from the region (*B. nigrovittata* Drew), as well as weakly attracting several cue-responsive species (Fay, 2011). The discovery of zingerone was a breakthrough in male lure research. It was a compound of similar structure to cue-lure and ME that attracted both cue- and ME-responsive species as well as non-responsive species.

Fay (2010) also field tested 50 commercially available benzene-ring compounds at one site in north Queensland over several months and found that some attracted cue- and ME-responsive species as well as two ‘non-responsive’ species. This showed that testing new compounds in areas of high fruit fly endemism can have unexpected results. The discovery of zingerone as an alternative lure and results of field testing of other novel compounds in Australia have shown that lure response in fruit flies is more complex than the previously held belief that fruit flies either respond to cue-lure or ME or are non-responsive.

In Papua New Guinea (PNG) there are three pest species of biosecurity concern to Australia that do not respond to cue-lure or methyl eugenol: the cucurbit pests *B. atrisetosa* (Perkins) and *B. decipiens* (Drew) and guava pest *B. obliqua* (Malloch). In Australia there are two non-responsive species of concern, *B. cucumis* (French) the cucumber fly, and *B. halfordiae* (Tryon) a pest of market access concern due to old host records from citrus (May, 1953).

This study aimed to determine the response of Dacini fruit flies to unproven eugenol analogues and zingerone in Papua New Guinea and Australia, particularly to determine if they would be attractive to pest species that are non-responsive to cue-lure or ME.

## Methods

Six lures were used at each site: isoeugenol (CAS 97-54-1), methyl-isoeugenol (CAS 93-16-3), dihydroeugenol (CAS 2785-87-7), zingerone (CAS 122-48-5), cue-lure (CAS 3572-06-3) and methyl eugenol (CAS 95-15-2). Lures were made from dental wick and dosed at 3 mL lure to 1 mL malathion. All lures were sourced from Sigma Aldrich, Castle Hill, New South Wales. Lures were placed in Steiner traps hung approximately 1.3 m from the ground in shady trees that were fruiting or near fruiting trees. Traps were placed a minimum of five metres apart. Lures were replaced every eight weeks. Traps were cleared weekly except over winter when they were cleared fortnightly due to cooler, drier weather and resultant lower trap catches. Flies were identified under a stereomicroscope using Drew (1989). Sites were chosen to represent a diversity of habitats (rainforest, transition forest between rainforest and sclerophyll, beachside, urban, savannah with orchards). Trapping locations were in the lowlands near Cairns, highlands on the Atherton Tablelands, Lockhart River region on Cape York Peninsula in Australia; and in PNG at the National Agricultural Research Institute (NARI) at Laloki in the National Capital District, and NARI Kerevat in East New Britain Province.

Statistical analysis of Australian data: each weekly trap catch was considered as an independent experimental unit. These discrete (count) data were analysed using a generalised linear model (McCullagh & Nelder, 1989) with the Poisson distribution and log link, using GenStat (2013). In this model the standard errors were associated with, and appropriate for, the fitted means. An over-dispersed Poisson model was adopted for the species which displayed this feature. The interaction between 'location', 'month' and 'year' was fitted first (to account for patterns in abundance), followed by 'lure'. Adjusted means were estimated, and significant differences between these were obtained using protected least significant difference (LSD) testing. Not all PNG samples had been collated at the time of writing so statistical analysis comparing cue-lure and ME trap catches with the novel lures was not possible.

## Results

Of the pest or market access species *B. halfordiae* and *B. obliqua* showed some response to the new eugenol analogs. *Bactrocera cucumis* did not respond to the new lures but in another component of this project was attracted to cucumber volatile lure, a lure developed in Hawaii as a female biased attractant for melon fly *B. cucurbitae* (Coquillett) (Siderhurst & Jang, 2010; Royer et al., 2014). In PNG *B. atrisetosa* and *B. decipiens* did not respond to the new lures but the latter was trapped several times at the protein Cera Trap®.

## Australia

317,908 fruit flies representing 49 species were collected from 2978 trap clearances between March 2012 and May 2014 (Table 1 for all species trapped at the lures tested and Table 2 for a summary of mean trap catch for each species with occurrence greater than 1% in all trap clearances, showing significant differences between species.).

Of the 49 species collected twenty species of *Bactrocera* and five species of *Dacus* were trapped at cue-lure and 11 species of *Bactrocera* and one species of *Dacus* were trapped at ME. Three species were recorded for the first time at a male lure (at the eugenol analogues) and two new species were collected at zingerone.

### *Eugenol analogues summary*

Dihydroeugenol attracted four non-responsive species, five ME-responsive species and three cue-responsive species. This lure was significantly more attractive to *B. visenda* than isoeugenol and methyl-isoeugenol and was roughly 10% as attractive as ME to this species (see Tables 1 and 2).

Isoeugenol attracted five non-responsive species, seven ME-responsive species and two cue-responsive species.

Methyl-isoeugenol attracted three non-responsive species, ten ME-responsive species and one cue-responsive species. It was significantly more attractive to the ME-responsive *B. cacuminata*, *B. laticaudus*, *B. musae*, *B. pallida* and *B. yorkensis* than isoeugenol or dihydroeugenol and was nearly half as attractive as ME to *B. pallida* (Table 2).

### *Zingerone summary*

Zingerone attracted seven non-responsive species, four of which had previously been recorded at zingerone (Fay 2011), and 13 cue-responsive species.

A new undescribed species, *D. sp. nr. pusillus*, closely related to the ME-responsive *D. pusillus* (May), was trapped in the Lockhart River region on 31 occasions, sometimes in high numbers (e.g. 63 flies in one trap clearance) with a total of 259 flies trapped. A single fly of another new undescribed species of *Dacus* was also collected at Lockhart River in November 2013. These species will be described in another paper (Royer JE & Hancock DL, unpublished data).

### *Non-responsive' species responding to new lures*

Three species of *Bactrocera* were recorded for the first time at a male lure during the trials (*B. halfordiae*, *B. barringtoniae* and *B. bidentata*, all recorded at one or more of the eugenol analogues). One species, *B. murrayi*, was recorded for the first time at methyl-isoeugenol and dihydroeugenol.

*Bactrocera halfordiae* (Tryon) was significantly more attracted to isoeugenol (mean 0.20) than to dihydroeugenol (mean 0.01) and did not respond to any other lure.

**Table 1.** Fruit fly species trapped at eugenol analogues, zingerone, methyl eugenol and cue-lure in north Queensland.

Species	Lure response	Methyl isoeugenol	Isoeugenol	Dihydro eugenol	Zingerone	Methyl eugenol	Cue-lure
<i>B. aberrans</i> (Hardy)	none		1				
<i>B. abscondita</i> (Drew & Hancock)	cue						3
<i>B. aeruginosa</i> (Drew & Hancock)	cue				1		3
<i>B. aglaiae</i> (Hardy)	zing				3		
<i>B. alyxiae</i> (May)	cue				2		3
<i>B. antigone</i> (Drew & Hancock)	cue						3
<i>B. aurea</i> (May)	zing				1		
<i>B. amplexiseta</i> (May)	ME					3	
<i>B. bancroftii</i> (Tryon)	ME <sup>w</sup>					1	
<i>B. barringtoniae</i> (Tryon)	none	3	2	1			
<i>B. bidentata</i> (May)	none	2	3	3			
<i>B. breviaculeus</i> (Hardy)	cue				1		3
<i>B. bryoniae</i> (Tryon)	cue				1		3
<i>B. cacuminata</i> (Hering)	ME	2	1	1		3	
<i>B. chorista</i> (May)	cue						3
<i>B. endiandrae</i> (Perkins & May)	ME	1				3	
<i>B. fagraea</i> (Tryon)	cue			1			3
<i>B. fallacis</i> (Drew)	cue						1
<i>B. frauenfeldi</i> (Schiner)	cue				1		3
<i>B. halfordiae</i> (Tryon)	none		3	1			
<i>B. jarvisi</i> (Tryon)	zing				3		1
<i>B. kraussi</i> (Hardy)	cue	2	3	1			2
<i>B. laticaudus</i> (Hardy)	ME	1	1			3	
<i>B. manskii</i> (Perkins & May)	cue						3
<i>B. mayi</i> (Hardy)	ME	1				3	
<i>B. melanothoracica</i> Drew	ME	1	1			3	
<i>B. murrayi</i> (Perkins)	none	3	2	1			
<i>B. musae</i> (Tryon)	ME	2	1	1		3	
<i>B. neohumeralis</i> (Hardy)	cue				1		3
<i>B. pallida</i> (Perkins & May)	ME	2	1	1		3	
<i>B. peninsularis</i> (Drew & Hancock)	cue						1
<i>B. quadrata</i> (May)	cue						3
<i>B. russeola</i> (Drew & Hancock)	cue						1
<i>B. silvicola</i> (May)	cue		1	1	1		3
<i>B. speewahensis</i> Fay & Hancock	zing				3		
<i>B. strigifinis</i> (Walker)	cue						3
<i>B. tigrina</i> (May)	none				1		
<i>B. tryoni</i> (Froggatt)	cue				1		3
<i>B. unirufa</i> Drew	ME	1				3	
<i>B. visenda</i> (Hardy)	ME	1	1	2		3	
<i>B. yorkensis</i> Drew & Hancock	ME	3	2	2			
<i>D. absonifacies</i> (May)	cue				3		2
<i>D. aequalis</i> Coquillett	cue				2		3
<i>D. axanus</i> (Hering)	cue				2		2
<i>D. bellulus</i> Drew & Hancock	cue				1		3
<i>D. pusillus</i> (May)	ME					2	
<i>D. secamoneae</i> Drew	cue				3		2
<i>D. sp. nr. pusillus</i>	none				3		
<i>D. sp. nov.</i>	none				1		

1= weak attraction, 2= moderate attraction, 3= strong attraction, superscript W = weakly attracted

**Table 2.** Mean weekly trap catches of fruit fly species at different lures in north Queensland, showing significant differences between lures.

	Cue-lure	Methyl eugenol	Zingerone	Isoeugenol	Methyl- isoeugenol	Dihydro eugenol
<i>B. abscondita</i>	1.217 a	0.000 b	0.000 b	0.000 b	0.000 b	0.000 b
<i>B. aeruginosa</i>	3.261 a	0.000 b	0.003 b	0.000 b	0.000 b	0.000 b
<i>B. aglaiae</i>	0.000 a	0.000 a	1.676 b	0.000 a	0.000 a	0.000 a
<i>B. alyxiae</i> <sup>x</sup>	46.156 a	0.000 b	0.077 c	0.000 b	0.000 b	0.000 b
<i>B. amplexiseta</i>	0.000 a	0.584 b	0.000 a	0.000 a	0.000 a	0.000 a
<i>B. barringtoniae</i>	0.000 a	0.000 a	0.000 a	0.093 b	0.136 c	0.041 d
<i>B. bidentata</i>	0.000 a	0.000 a	0.000 a	0.018 b	0.006 c	0.025 d
<i>B. breviaculeus</i> <sup>x</sup>	4.385 a	0.000 b	0.038 c	0.000 b	0.000 b	0.000 b
<i>B. bryoniae</i>	1.071 a	0.000 b	0.005 c	0.000 b	0.000 b	0.000 b
<i>B. cacuminata</i> <sup>x</sup>	0.000 a	19.407 b	0.000 a	0.137 c	0.600 d	0.057 e
<i>B. chorista</i>	0.064 a	0.000 b	0.000 b	0.000 b	0.000 b	0.000 b
<i>B. endiandrae</i> <sup>x</sup>	0.000 a	70.781 b	0.000 a	0.000 a	0.483 c	0.000 a
<i>B. fagraea</i>	0.107 a	0.000 b	0.000 b	0.000 b	0.000 b	0.001 b
<i>B. frauenfeldi</i> <sup>x</sup>	32.470 a	0.000 b	0.106 c	0.000 b	0.000 b	0.000 b
<i>B. jarvisi</i> <sup>x</sup>	0.011 a	0.000 a	18.422 b	0.000 a	0.000 a	0.000 a
<i>B. halfordiae</i>	0.000 a	0.000 a	0.000 a	0.202 b	0.000 a	0.008 c
<i>B. kraussi</i>	0.095 a	0.000 b	0.000 b	0.502 c	0.108 a	0.046 d
<i>B. laticaudus</i>	0.000 a	0.437 b	0.000 a	0.003 c	0.013 d	0.000 a
<i>B. manskii</i>	2.059 a	0.000 b	0.000 b	0.000 b	0.000 b	0.000 b
<i>B. mayi</i>	0.000 a	1.100 b	0.000 a	0.000 a	0.018 c	0.000 a
<i>B. murrayi</i>	0.000 a	0.000 a	0.000 a	1.024 b	1.366 c	0.209 d
<i>B. musae</i>	0.000 a	14.890 b	0.000 a	0.198 c	0.674 d	0.117 e
<i>B. neohumeralis</i> <sup>x</sup>	71.680 a	0.000 b	0.090 c	0.000 b	0.000 b	0.000 b
<i>B. pallida</i>	0.000 a	1.587 b	0.000 a	0.242 c	0.763 d	0.025 e
<i>B. quadrata</i>	0.313 a	0.000 b	0.000 b	0.000 b	0.000 b	0.000 b
<i>B. silvicola</i>	1.462 a	0.000 b	0.001 b	0.005 b	0.000 b	0.001 b
<i>B. strigifinis</i>	0.871 a	0.000 b	0.000 b	0.000 b	0.000 b	0.000 b
<i>B. tryoni</i> <sup>x</sup>	27.806 a	0.000 b	0.062 c	0.000 b	0.000 b	0.000 b
<i>B. unirufa</i>	0.000 a	0.109 b	0.000 a	0.000 a	0.001 a	0.000 a
<i>B. visenda</i>	0.000 a	1.817 b	0.000 a	0.006 c	0.027 d	0.283 e
<i>B. yorkensis</i>	0.000 a	0.000 a	0.000 a	0.050 b	0.124 c	0.026 b
<i>D. absonifacies</i>	0.048 a	0.000 b	0.243 c	0.000 b	0.000 b	0.000 b
<i>D. aequalis</i>	0.469 a	0.000 b	0.169 c	0.000 b	0.000 b	0.000 b
<i>D. bellulus</i>	0.193 a	0.000 b	0.002 bc	0.000 b	0.000 b	0.000 b
<i>D. secamoneae</i>	0.009 a	0.000 b	0.097 c	0.000 b	0.000 b	0.000 b

Means in the same column with a letter in common are not significantly different ( $p>0.05$ ).

Species without an 'x' are those with under-dispersal and standard Poisson GLM was used.

<sup>x</sup>Species with an 'x' are those with over-dispersal and over-dispersed Poisson GLM used.

*Bactrocera barringtoniae* (Tryon), *B. bidentata* (May) and *B. murrayi* (Perkins) all responded to isoeugenol, methyl-isoeugenol and dihydroeugenol. *Bactrocera barringtoniae* was significantly more attracted to methyl-isoeugenol (mean 0.14) than to isoeugenol (mean 0.09) or dihydroeugenol (mean 0.04). *Bactrocera bidentata* was significantly more attracted to dihydroeugenol (mean 0.03) and isoeugenol (mean 0.02) than to methyl-isoeugenol (mean 0.01). *Bactrocera murrayi* was significantly more attracted to methyl-isoeugenol (mean 1.37) than isoeugenol (mean 1.02) or dihydroeugenol (mean 0.21).

#### *Cue- or ME-responsive species that were more responsive to new lures*

*Bactrocera kraussi* (Hardy), a cue-responsive species, was significantly more attracted to isoeugenol (mean 0.50) than to cue-lure (mean 0.10). There was no significant difference between its attraction to cue-lure and methyl-isoeugenol (mean 0.11). It was also less attracted to dihydroeugenol (mean 0.05).

*Bactrocera yorkensis* Drew & Hancock, a ME-responding species (Drew *et al.* 1999), did not respond to ME at all during this study but responded to all three of the eugenol analogues. It was significantly more attracted to methyl-isoeugenol (mean 0.12) than isoeugenol (mean 0.05) and dihydroeugenol (mean 0.03).

Zingerone was significantly more attractive to two cue-responsive *Dacus* species than cue-lure: *D. absonifacies* (May) (zingerone mean 0.24, cue-lure mean 0.05) and *D. secamoneae* Drew (zingerone mean 0.10, cue-lure mean 0.01).

### **Papua New Guinea**

The new lures trapped cue- and ME-responsive species and non-responsive species (Table 3).

*Bactrocera obliqua*, a target non-responsive minor pest, was detected twice at isoeugenol and once at methyl-isoeugenol. *Bactrocera aglaiae* and *B. barringtoniae* were recorded in PNG for the first time (at zingerone and the eugenol analogues respectively).

#### *Eugenol analogues summary*

Dihydroeugenol trapped two non-responsive species, two ME-responsive species, one cue-responsive species and an unknown species of *Bactrocera* (Table 3).

Isoeugenol trapped three non-responsive species, two ME-responsive species and one unknown species of *Bactrocera*.

Methyl-isoeugenol trapped three non-responsive species, eight ME-responsive species, one cue-responsive species and an unknown species of *Bactrocera*. This lure attracted the most ME-responsive species and in higher numbers than the other eugenol analogues.

**Table 3.** Fruit fly species trapped at eugenol analogues, zingerone, methyl eugenol and cue-lure in Papua New Guinea.

	Lure response	Methyl isoeugenol	Isoeugenol	Dihydro- eugenol	Zingerone	Methyl eugenol	Cue-lure
<i>B. abdonigella</i> (Drew)	cue						1
<i>B. anthracina</i> (Drew)	cue						1
<i>B. atramentata</i> (Hering)	cue			1	2		3
<i>B. aglaiae</i> (Hardy)	zing				1		
<i>B. barringtoniae</i> (Tryon)	none	1	2	1			
<i>B. breviaculeus</i> (Hardy)	cue						1
<i>B. bryoniae</i> (Tryon)	cue				1		2, 3
<i>B. cucurbitae</i> (Coquillett)	cue	1			1, 1		3, 3
<i>B. curta</i> (Drew)	cue						2
<i>B. dorsalis</i> (Hendel)	ME	2	1	1			3
<i>B. frauenfeldi</i> (Schiner)	cue						3
<i>B. fulvicauda</i> (Perkins)	ME	2				3	
<i>B. lampabilis</i> (Drew)	ME					2	
<i>B. moluccensis</i> Perkins	cue				1		2, 3
<i>B. murrayi</i> (Perkins)	none	2	1	1			
<i>B. musae</i> (Tryon)	ME	2, 1	1	1		3, 3	
<i>B. neohumeralis</i> (Hardy)	cue						1
<i>B. neonigrita</i> Drew	ME						1
<i>B. nigrescens</i> (Drew)	ME						3
<i>B. nigrescentis</i> (Drew)	cue						1
<i>B. obliqua</i> (Malloch)	none	1	1				
<i>B. reflexa</i> (Drew)	cue						2
<i>B. seguyi</i> (Hering)	ME	1				2	
<i>B. speculifera</i> (Walker)	ME	1				2, 2	
<i>B. triangularis</i> (Drew)	cue				1		3
<i>B. trivialis</i> (Drew)	cue				1		3
<i>B. umbrosa</i> (Fabricius)	ME	2, 2				3, 3	
<i>B. unistriata</i> (Drew)	ME	1				2	
<i>B. visenda</i> (Hardy)	ME	1				1	
<i>B. (B.)</i> sp. nr. <i>anomala</i>	none				1		
<i>B. (B.)</i> sp. nr. <i>pallida</i>	none	2	1	1		2	
<i>B. (B.)</i> sp. nr. <i>dyscrita</i>	none				3		
<i>B. (T.)</i> sp. n.	none				1, 2		
<i>D. axanus</i> (Hering)	cue						1, 1
<i>D.</i> sp. nr. <i>impar</i>	none				1		
<i>D.</i> sp. nr. <i>pusillus</i>	none				1		

1 = weak response, 2 = moderate response, 3 = strong response; Laloki = standard numerals, Kerevat = italicised numerals.

### Zingerone summary

Zingerone trapped six cue-responsive species, one species previously recorded at zingerone in Australia (*B. aglaiae*) (Fay, 2011) and three unknown species of *Bactrocera* and two of *Dacus*.

### Discussion

These data are the first records of *B. halfordiae*, *B. barringtoniae* and *B. bidentata* regularly responding to male lures and *B. murrayi* responding to methyl-isoeugenol and dihydroeugenol (Royer, 2015). Fay (2010) recorded *B. murrayi* at isoeugenol only, but in this study it was more attracted to methyl-isoeugenol.

*Bactrocera halfordiae* responded strongly to isoeugenol and weakly to dihydroeugenol. These lures were also tested in southeast Queensland from September to November 2014 in rainforest where *Planchonella australis* (Sapotaceae), the preferred host of *B. halfordiae*, was common. Over an eight week period this species was regularly trapped, with an average weekly catch of 48 flies at isoeugenol and two flies at dihydroeugenol.

*Bactrocera halfordiae* has recently been considered a market access pest due to old host records from citrus (May, 1953). However, since then the only host records have been in rainforest fruit (Hancock et al., 2000). A lure for *B. halfordiae* has importance if there is ever a requirement to monitor populations to demonstrate pest freedom for market access.

*Bactrocera obliqua* responded weakly to isoeugenol and methyl-isoeugenol in PNG. This weak attraction may give a clue that a structurally similar compound may be a more effective lure. This has been the case for several other species that have been recorded sporadically at a lure and found to be strongly attracted to a structurally similar lure. For example, *B. jarvisi* was known to be weakly attracted to cue-lure (Drew, 1989; Royer & Hancock, 2012) and later found to be strongly attracted to zingerone (Fay, 2011).

*Bactrocera kraussi*, a cue-responsive species, responded more strongly to isoeugenol than cue-lure and there was no significant difference in its attraction to cue-lure and methyl-isoeugenol. Although *B. kraussi* has a wide host range and is considered a minor pest it is relatively rare in cue-lure trap catches (Royer JE, unpublished data), and the data presented here indicates that this is due to a weak cue-lure attraction. This species is of some economic concern as it has been recorded from banana, citrus, guava, mango and peach (Hancock et al., 2000). Having a stronger attractant for it would be useful for horticultural production areas wishing to demonstrate pest freedom or improve control through male annihilation.

Other cue- or ME-responsive species were more attracted to the new lures. The cue-responsive *D. absonifacies* and *D. secamoneae* were significantly more attracted to zingerone than to cue-lure. The ME-responsive *B. yorkensis* (Drew et al., 1999) was not trapped at ME at all during this study but responded to the eugenol analogues. It was most attracted to methyl-isoeugenol and then to isoeugenol and dihydroeugenol. As several lure-responsive



species in this study were found to be more attracted to new lures, it is plausible that there may be more attractive lures for other species in different geographic regions, perhaps even pest species.

Methyl-isoeugenol trapped more ME-responsive species (and in higher numbers) and zingerone attracted more cue-responsive species than the other lures in both countries. Methyl-isoeugenol is structurally similar to ME and zingerone to cue-lure so these similarities in attraction are not surprising.

Several new distributions were recorded for ‘non-responsive’ species in Australia and PNG. With the discovery of attractive lures it is expected that species will be detected in new locations. In Australia *B. agalae*, *B. aurea*, *B. bidentata*, *B. halfordiae*, *B. speewahensis*, *D. absonifacies* and *D. secamoneae* were recorded either for the first time or more widely in north Queensland at zingerone or the eugenol analogues. *Bactrocera aglaiae* and *B. barringtoniae* were recorded for the first time in PNG at zingerone and the three eugenol analogues respectively. These two species were previously known from northern Queensland (Hancock et al., 2000). The hosts of *B. aglaiae* (*Aglaia sapindina*) and *B. barringtoniae* (*Barringtonia calypttrata*) both occur in PNG (ATRP, 2014) so it is not surprising to find that they occur there.

In summary, new male attractants – zingerone, isoeugenol, methyl-isoeugenol and dihydroeugenol - were tested widely for the first time in northern Australia and PNG. This resulted in the first records at male lures of the PNG guava pest *B. obliqua*, and the Australian species *B. halfordiae*, *B. barringtoniae* and *B. bidentata*, all of which were recorded at the eugenol analogues. Several ME- and cue-responsive species were more attracted to the new lures than their respective lures, including the Australian pest *B. kraussi* which was more attracted to isoeugenol than cue-lure. New species distributions and new undescribed species were also recorded. This work highlights the value of testing new lures in areas of high fruit fly diversity where multiple species responses have the potential for new findings on species attractions and lure activity.

## Acknowledgements

This research was funded by the Australian Centre for International Agricultural Research under the project PC2012/053 *Feasibility study on novel lures for pest fruit flies that do not respond to known male attractants*. Thanks to Dr Harry Fay for providing the impetus for this project, John Bokosou, Kiteni Kurika, Sharon Agovaua, Amanda Mararuai and Benjamin Niangu for work conducted in PNG, Dr David Mayer for statistical analysis of the data, Rose Laughton and Linda Baker for field work and assistance with identification of fruit flies in Australia, and John Pritchard, and Jack and Sue Hasenpusch for trapping conducted at Lockhart River and Garradunga.

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## Field evaluation of attractive lures for *Bactrocera minax* (Enderlein) (Diptera:Tephritidae), for use in bait sprays in Tsirang, Bhutan

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### Abstract

**Background:** The Chinese citrus fruit fly, *Bactrocera minax* (Enderlein) is a major univoltine fruit fly pest of mandarin in Bhutan. In order to provide an environmentally friendly alternative to insecticidal cover sprays lures, that can potentially be used as baits in spot sprays, were field tested for their effectiveness in attracting and killing *B. minax* flies.

**Methods:** Field trials were conducted from May to August, 2013, in four citrus orchards in the Tsirang District, Bhutan. The attractiveness of four lures, Probiofer L (Protein Hydrolysate Liquid), Probiofer A (Protein Hydrolysate Powder), Pinnacle protein (Mauri Pinnacle protein®) and Jaggery (a concentrated product of sugar cane), were determined through weekly counts of *B. minax* captured in PET bottle traps containing these mixtures. Each treatment was replicated six times and placed in one of four citrus blocks (96 traps in total). Female flies ensnared in the traps were dissected to determine their sexual maturity. The attraction and mortality of *B. minax* to Probiofer L, Probiofer A and Pinnacle protein (all treatments included the toxicant malathion), when applied as spot sprays, were determined by counting dead flies collected on white sheets placed below the treated spots, 1h after application (three replicates per treatment).

**Results:** Among the lures, Probiofer L and Pinnacle protein were the most attractive, capturing significantly more flies compared to Probiofer A and Jaggery. In attraction and mortality trials with spot sprays, flies attracted to Pinnacle protein had the highest mortality followed by Probiofer A, Probiofer L and a water control. Higher numbers of female *B. minax* were attracted to all lures compared with male flies. Female *B. minax* flies that were dissected found that the majority of the flies were immature and semi-mature till the first week of June, with most flies attaining full sexual maturity by the end of June. Peak fly activity was observed in the second and third week of June.

**Conclusions:** Probiofer L, Probiofer A and Pinnacle protein baits hold promise to be used as lures for spot sprays in managing this pest.

**Keywords:** attraction, *Bactrocera minax*, Chinese citrus fruit fly, protein bait.

## Introduction

The Chinese citrus fruit fly, *Bactrocera minax* (Enderlein) (Diptera:Tephritidae) is a unique univoltine fly recorded mostly in the temperate regions of southern China, India (West Bengal and Sikkim) and Bhutan (Drew, 1979; Wang & Luyi, 1995). The host range is limited to species of citrus (Allwood et al., 1999). In Bhutan, it is a significant pest of mandarine (*Citrus reticulata* Blanco) causing over 50% yield loss (Dorji et al., 2006).

The existing control strategies recommend the use of fortnightly protein bait spray applications from early May to late July and/or systemic insecticide cover sprays in early July, plus the removal of fallen fruit every 10 days (Dorji et al., 2006). In Bhutan, cover sprays are being discouraged due to a shift in government policies that advocate reduced pesticide agricultural practices. In addition, globally, the use of cover sprays in fruit fly control programs is losing popularity because of residues in fruit, human health concerns and impacts on beneficial and non-target organisms (Vijayasegaran, 1994). Although cover sprays provide good fruit fly control (Allwood, 1997), protein bait sprays are the most widely used strategy as this technique reduces the total amount of insecticide used with minimum impact on beneficial insects and the environment (Prokopy et al., 1992; Chueca, 2008).

To date, there have not been any published studies that have assessed the attractancy and effectiveness of various protein-based lures against *B. minax* in Bhutan. This information is critical as the choice of a protein lure in a bait spray could impact the success of a programme (Fabre et al., 2003).

In this study, the main objective was to determine the attraction and effectiveness of various protein-based lures against *B. minax*. Specifically, we determined the attractiveness of four lures, Probiofer L (protein hydrolysate liquid), Probiofer A (protein hydrolysate powder), Pinnacle protein (Pinnacle protein®) and Jaggery (a concentrated product of sugar cane) contained within PET bottle traps, to *B. minax*. The sexual maturity of female flies captured was determined. The attraction and mortality of Probiofer L, Probiofer A and Pinnacle protein against *B. minax*, when applied as spot sprays mixed with malathion, were also determined.

## Material and Methods

### Field Sites

Field trials were conducted from May-August, 2013, in citrus orchards in the Tsirang District (90°.12'E, 27°.01'N, Altitude: 1200m) of central Bhutan. The orchards comprise mainly mandarin (*Citrus reticulata* Blanco), and few trees comprising of other Rutaceae family such as lime (*Citrus latifolia* Tanaka) and lemon (*Citrus limon* L). No pest management practices were carried out throughout the experimental period.

### *Lures*

The lures consisted of the following mixtures: 1) Probiofer L (Chaitanya Chemicals, New Delhi, India): 50ml solution in 1 L water, 2) Probiofer A (Chaitanya Chemicals, New Delhi, India): 5g in 1 L water 3) Pinnacle protein (Australia Pvt Limited, Toowoomba, Queensland, Australia): 50ml Mauri Pinnacle protein® in 1 L water and 4) Jaggery (concentrated product of sugar cane): 100g mixed in 1 L water. The label rates were followed as recommended.

#### *Trial 1: Determination of attractiveness of different lures against B. minax*

Trial 1 was conducted in four citrus orchards, located within close proximity (30-50 m) of one another. The trial ran from 8 May 2013 until 28 August 2013. Four lures, Probiofer L, Probiofer A, Pinnacle protein and Jaggery were evaluated for their attractiveness against *B. minax* using modified PET bottle traps (height: 20 cm, diameter: 10 cm, with two, 2x4 cm, cuts 5 cm from the bottom of the bottle for fly entry). Each PET bottle traps were filled with 150ml, of one of each lure, which attracted the flies that were then drowned in the lure. Traps were suspended 1.5m above the ground. In total, 96 traps were used each week. The traps were randomly placed, with a distance of 10m maintained between individual traps, in each orchard. On a weekly interval, counts of *B. minax* captured in PET bottle traps were recorded. The traps were then serviced, refilled with fresh lures and placed in new positions within the orchard. Treatments were replicated six times within each orchard. The trapped flies were preserved in 70% alcohol, counted and sexed. The female flies were dissected to determine their sexual maturity and classified as mature (presence of mature eggs), semi mature (ovaries with developing eggs, but not fully mature), and immature flies (eggs not present) using the scoring system of Fletcher et al. (1978).

#### *Trial 2: Attractancy and mortality of lures against B. minax, when applied as spot sprays.*

Trial 2 was conducted in a citrus orchard in Tsirang, Bhutan and was repeated on two occasions, from 11-16 May 2013 and 20-25 May, 2013. Three lures, Probiofer L, Probiofer A and Pinnacle protein were mixed with malathion 50 EC insecticide (Rallis India Limited) at the rate of 2 mL/L solution. Water was used as control. A spot spray of 50mL per tree was carried out with a hand held sprayer (500mL capacity). Each lure-insecticide mixture was replicated three times, and a distance of 10m maintained between the treated spots. Below each treated spot, a white cloth sheet (3mx3m) was placed to collect the dead flies. Dead flies were collected up to one hour after the lure-insecticide mixture application.

Data analyses were carried out using IBM SPSS Statistics (IBM SPSS Statistics, 2010). Prior to analysis, normal distribution was tested using the Kolmogorov-Smirnov test and homogeneity of variances was tested using the Levene's test. The data were log transformed [ $\log(x+1)$ ] to stabilize variances and to normalize the data. For Trial 1 the total number of male and female *B. minax* flies captured in each treatment was subjected to ANOVA with lure, sex and date as factors. Potential interactions among these factors were also tested. Differences in treatment means were considered statistically significant at the  $P = 0.05$  level. For Trial 2 the analysis procedure followed was similar to Trial 1. The total number of male

and female *B. minax* flies, obtained from each of the lure-insecticide mixtures, was subjected to a two-way ANOVA with lure and sex as factors. Differences in treatment means were considered statistically significant at the  $P = 0.05$  level.

## Results

In trial 1, a total of 679 flies (631 females and 48 males) were captured. The lures had a significant effect on the number of flies trapped ( $F_{3,192} = 4.697$ ,  $P = 0.003$ ). Fly captures in lures were different between male and female flies with significantly more females captured than males ( $F_{1,192} = 22.785$ ,  $P = 0.000$ ). A significant sex  $\times$  lure interaction ( $F_{3,192} = 3.868$ ,  $P = 0.010$ ) was observed with lures varying in capturing male and female flies. For the treatment  $\times$  sex interaction with females, the lures Probiofer L and Pinnacle protein were the most attractive, capturing significantly more flies compared to Probiofer A and Jaggery (Table 1).

No significant difference was observed in female attraction between Probiofer A and Jaggery (Table 1). Jaggery was the least attractive lure compared to the other three lures (Table 1). For males, there were no significant differences among lures (Table 2).

**Table 1.** Mean number of female *Bactrocera minax* captured in PET bottle traps containing different lures.

Treatment	Mean flies captured
Pinnacle protein	45.12a
Probiofer L	42.64a
Probiofer A	11.52b
Jaggery	5.44b

Means followed by the same letter do not differ significantly. (Fisher's LSD test on log ( $x+1$ ) transformed data;  $P < 0.05$ )

**Table 2.** Mean number of male *Bactrocera minax* captured in PET bottle traps containing different lures.

Treatment	Mean flies captured
Pinnacle protein	1.89a
Probiofer L	1.65a
Probiofer A	1.32a
Jaggery	0.29a

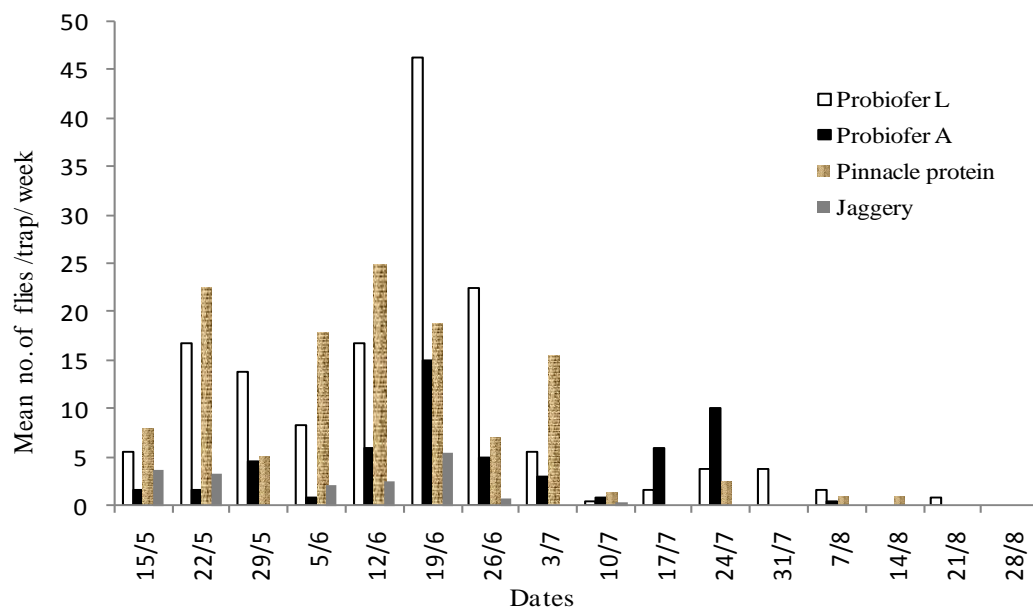
Means followed by the same letter do not differ significantly. (Fisher's LSD test on log ( $x+1$ ) transformed data;  $P < 0.05$ )

Fly captures varied with date, with peak activity recorded on the 19 June 2013, with no fly activity occurring on 28 August 2013 (Fig.1). Female *B. minax* flies that were dissected indicated that the majority of trapped flies were immature and semi-mature until the first

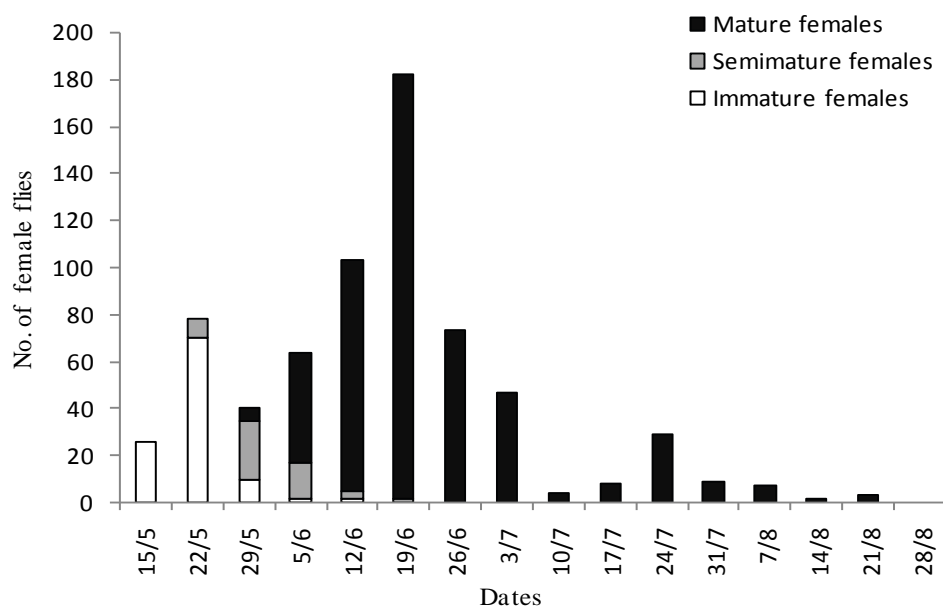


week of June, with most trapped flies sexually mature throughout June, July and August (Fig. 2).

In trial 2, attraction and mortality of *B. minax* was highest for spot sprays of Pinnacle protein followed by Probiofer A, Probiofer L and the control ( $F_{3,40} = 9.713$ ,  $P = 0.000$ ) (Table 3). No significant differences were observed among Probiofer A, Probiofer L and the control (Table 3).



**Fig. 1.** Mean number of *Bactrocera minax* captured per trap weekly, using various lures (Probiofer L, Probiofer A, Pinnacle protein and Jaggery) in experimental orchards in Tsirang, Bhutan.



**Fig. 2.** Pattern of seasonal of ovarian development for female *Bactrocera minax* captured in PET bottle traps in a mandarin orchard at Tsirang Bhutan in 2013.

**Table 3.** Mean number of *Bactrocera minax* killed with protein baits applied as spot sprays on mandarin trees.

Treatment	Mean flies dead
Pinnacle protein	23.26a
Probiofer L	4.00b
Probiofer A	6.63b
Control	0.00b

Means followed by the same letter do not differ significantly. (Fisher's LSD test on log (x+1) transformed data;  $P < 0.05$ )

## Discussion

Fruit fly control using insecticidal cover sprays have been associated with adverse effect on non-target and beneficial arthropods (Vijaysegaran, 1994) and its use in managing univoltine temperate tephritid species like *Rhagoletis* sp. is quite common (Aliniaze, 1984; Prokopy et al., 1990; Yee, 2007). Despite the fact that the use of cover sprays against *B. minax* is minimum in Bhutan, there is a need to test potential lures for use in protein bait sprays as an alternative to cover sprays against this pest.

The results of this study indicate that some of these lures tested such as the Pinnacle protein and Probiofer L can be considered for use in bait spot sprays against *B. minax*. For fly population monitoring, the results indicate that both Pinnacle protein and Probiofer L may be employed, as these lures elicited the same level of attraction for *B. minax*. However, the Pinnacle protein was the most effective lure both in terms of attracting the flies and its efficacy when applied as a bait spray. More female flies compared to males were attracted to and ensnared in the traps with lures. The results obtained here align with studies evaluating lures where stronger attraction to protein lures were reported in females than males (Malo, 1992; Vargas et al., 2002; Vargas & Prokopy, 2006) a response due to females requiring protein to fulfill their need for ovarian and sexual maturity (Hagen & Finney, 1950).

The lure Jaggery was discarded from the bait spot trial because it attracted limited number of *B. minax* flies compared to other lures in the attraction test. Jaggery is recommended as an effective lure against *Bactrocera dorsalis* (Hendel) in mango, *Mangifera indica* L., as spot sprays in India (Singh et al., 2008). However, Jaggery was found to be the least attractive lure for *B. minax*, indicating a significant variation in responses among different tephritid pest species.

The study also investigated the female sexual maturity pattern that has not been previously documented for Tsirang, a major citrus in production zone in central Bhutan. A seasonal phenology study conducted in *B. minax* in western Bhutan showed most flies to be mature by the end of May with very little oviposition occurring until mid-June (Dorji et al., 2006). This study showed most flies to be mature by the first week of June (73% of the captured flies). However, compared to number of immature and semimature flies, the majority of flies ensnared in protein lure traps comprised of sexually mature flies, and most of the flies captured were trapped by the first week of July (Fig.2). A similar pattern was observed by

Dorji et al. (2006) in protein baited McPhail traps over two season, where the majority of the trapped flies were sexually mature. This trend indicates that, although flies attain sexual maturity, they remain protein hungry. This indicates that sexually mature flies continue to require protein for egg development (Mangan, 2003; Perez-Staples, 2007). Therefore, aligning with this pattern of sexual maturation, protein baiting in these area can commence from early May and continue till the end of July, after which minimum number of flies are attracted to protein based lures (Fig.2).

When baits were tested for their efficacy in attracting and killing flies, Pinnacle protein outperformed the other two protein-based lures (Probiofer A and Probiofer L). This may partly be because these other lures, unlike Pinnacle protein, are not specifically manufactured as fruit fly baits. However, these baits are inexpensive and locally available. Hence, future studies are needed to investigate techniques to enhance the field efficacy of these baits as lures or when applied as spots sprays against *B. minax*.

### Acknowledgements

The authors are grateful to the ACIAR (Australian Centre for International Agricultural Research) citrus project in Bhutan and the Department of Agriculture, MoAF, Bhutan for their support.

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## **Electroantennogram of *Ceratitis capitata* and field responses on *Bactrocera dorsalis* with Cera Trap**

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### **Abstract**

**Background:** Due to the economic impact of fruit flies, much attention has been focused on the development of trapping systems for detection and monitoring pests and more recently to provide successful pest control without the use of insecticides. This is due to the lately public demand for more benign control techniques. In this respect, the mass trapping technique attempts to provide successful pest control reducing the pest population in the plot by means of traps baited with a lure with or without a low dose of insecticide. Here we performed a multidisciplinary approach to study the response of *Ceratitis capitata* to one hydrolyzed protein based bait and we measured the efficacy of a mass trapping system on *Bactrocera dorsalis* in field trials.

**Methods:** Electrophysiological response of *C. capitata* and a mass trapping field trial was performed on *B. dorsalis* with a commercial mass trapping system (Cera Trap®). The electroantennography (EAG) were recorded from different volatile compound doses, for both sexes as well as considering the female age fly.

**Results:** The results corroborated that volatiles released by the protein bait are detected by the antennae of *C. capitata*. A higher collection period for the released volatiles from a constant bait volume does not increase the relative EAG signal. Furthermore, over a range of doses tested, EAG female response was significantly greater than male response and using a fixed dose of substrate, the maximum EAG amplitude was reached for the 4-days old female fly.

**Conclusions:** The field trial results show that the Cera Trap® mass trapping system provides a high level of captures of *B. dorsalis*, particularly female sex, and therefore it provides a good opportunity to the Integrated Pest Management strategies, as well as organic citrus and fruit harvest.

**Keywords:** attractant, electroantennography, enzymatic hydrolysis, liquid protein, mass trapping, protein bait.

## Introduction

The Mediterranean fruit fly, *Ceratitis capitata* and the oriental fruit fly, *Bactrocera dorsalis*, are serious agricultural pests. Traditional control programs of these pests have long been carried out using conventional insecticides through aerial and terrestrial treatments (Kendra et al., 2011). However, lately there is a public demand for more benign alternatives techniques due to the adverse effects of broad-spectrum insecticides on humans, non-target organisms and the environment (El-Sayed, 2006; Rössler, 1989). Additional concerns exist because of the potential development of insecticide resistance (Vontas, 2011; Magaña, 2008; Anonymous, 1999; Georgiou, 1976). In the search for alternative control techniques, the mass trapping technique attempts to provide successful pest control reducing the pest population in the plot by means of traps baited with a lure with or without a low dose of insecticide (Georgiou, 1986; Steiner, 1952). The key objective of mass trapping is to capture the maximum number of insects before they reproduce or cause damage to crops (El-Sayed, 2006). Effective trapping techniques require the use of lures that are able to attract fruit flies more effectively than natural foods sources (Lasa, 2014).

Due to the economic impact of pests, much attention has been focused on the development of trapping systems for detection and monitoring pests. Many of these lures are based on hydrolyzed protein formulas (Martínez-Ferrer, 2010; McPhail, 1939) considering that female flies need protein for full ovarian development and egg production (Epsky, 1993; Gow, 1954).

Cera Trap is a liquid bait lure consisting of enzymatic hydrolyzed protein free of insecticides that releases a series of volatile compounds, mostly amines and organic acids. The Mediterranean fruit fly (medfly) is strongly attracted, enters into the traps baited with the hydrolyzed protein and, being unable to escape, drowns in the liquid and dies (Lasa, 2014; 2013; Llorens, 2008).

The aim of our study was to perform a multidisciplinary approach to study the response of *C. capitata*, to the hydrolyzed protein based bait. EAG analysis was used to measure antennal sensitivity to volatile chemicals emitted from the hydrolyzed protein bait to further understand the electrophysiology that underline behavioral response. Moreover, the efficacy of the mass trapping system was measured in the field to assess the number of *Bactrocera dorsalis* fly catches as well as the gender.

## Materials and Methods

### *Insects*

Pupae of *C. capitata* were obtained from two laboratory colonies maintained at the Madrid Polytechnic University (Spain) and University of Pisa (Italy). After eclosion, adult flies were segregated by sex and were maintained in separate cages provided with sucrose, honey and water until they were tested 0-15 days after emergence. The room conditions consisted of a 12/12 h L/D photoperiod, temperatures  $25 \pm 2$  °C and humidity  $75 \pm 10\%$  RH.

### *Electrophysiological response*

Volatile chemicals were collected from three different batches from Cera Trap (250 ml, 3 replicates per batch) by using a dynamic headspace collection method followed by ultrasonic extraction. Samples were confined in a cylindrical glass chamber (4.5 cm diameter, 25 cm length) that was tightly closed using ground glass fittings and a clamp. A charcoal-filtered air stream (500 ml min<sup>-1</sup>) was pulled over the Cera Trap (CT) liquid and the headspace was collected for 30 to 360 min at room temperature (20 °C ± 2) on an adsorbent trap containing Tenax-TA or Tenax GC (60/80 mesh, Supelco). The in-line adsorbent trap consisted of a commercial GC glass liner for split/splitless injector Varian 1078/1079 (Reference 2637101, Supelco) of a 4.0 mm of diameter and filled with 100 mg of the above absorbents between two glass-wood plugs. The adsorbent trap was thermally activated before the collection into the chromatographic injector (Varian 3400 CX) where it was kept at 280°C ± 2 during 30 min. Volatile chemicals were eluted from the Tenax using 500 µL of HPLC hexane by sonication during 3 min. and the hexane extract was filtered and dried using anhydrous MgSO<sub>4</sub> and concentrated. Sample volumes were reduced to dryness under slow stream of N<sub>2</sub>. Samples were sealed and stored at - 40°C ± 2.

EAG responses were recorded with a Syntech Electroantennogram system (Hilversum, The Netherlands). Whole fly heads, with the antennae extended, were mounted between electrodes using conductive gel (Spectra 360, Parker Laboratories, USA) and placed under purified air flow (100 ml min<sup>-1</sup>). The best collected released volatiles were dissolved in 10 µL of HPLC grade hexane to form 10 extract equivalents (EE). From these solutions, 1 µL aliquots (containing 1 EE) were pipette onto glass-fiber papers, solvent was allowed to evaporate for ca. 30 seconds, and then the filter papers were inserted into Pasteur pipettes (15 cm long). Each test cartridge was loaded within 30 seconds of its preparation to the antenna of three replicate measurements. For each extract tested, EAGs were recorded from at least five flies of each sex. Hexane was used as control stimuli as it was the solvent used to dissolve different Cera Trap extracts and hexan-1-ol at a 10 µg (>98% pure, Sigma-Aldrich, Germany) was used as standard stimuli because its stimuli signal is well known. These controls were interspersed about every ten extracts tested. EAG responses were measured initially in units of mV, and then normalized to percent responses relative to the standard chemical reference (hexan-1-ol). EAG responses (mV) to a test extract were first adjusted by subtraction of response to accompanying control dividing the amplitude of the EAG responses were recorded from 76 insects and the response from each insect was the mean of three replicate measurements. For EAG temporal profiles the volatiles released by 250 ml of Cera Trap during 180 min and were collected by above mentioned conditions. The extract was diluted in 10 µl of hexane and 1 µl aliquots containing 1 EE were used in each puff.

### *Field trials*

Cera Trap is an attractant completely free of pesticides in its formulation and none is required in the traps. It is based on a liquid protein obtained by an exclusive method of enzymatic hydrolysis and has a strong attraction capacity for medfly. The system works thanks to a



regular emission of volatile compounds and as a result of that the fly strongly attracted, enters into the baited trap and being unable to escape drowns into the liquid and dies. A field trial was carried out in a mango orchard in Sri Prachen, Suphanburi (Thailand) during January – April 2009 to assess the number of *Bactrocera dorsalis* captures and gender of the flies. The traps were hung on the tree at 60 days after fruit setting (45-60 days approximately before the commercial ripening of the fruits). The trap density was approximately 50 traps per hectare. The traps were homogeneously placed in all the rows of the plot with the direction from East to West. The number of flies in the trap was counted at 1, 3, 5, 7, 14 and 28 days after being hung. A complete randomized block design with five replications was performed. Trap density was 50 traps ha<sup>-1</sup> and two treatments were compared: 1) standard farmer system (cotton wool + methyl eugenol + malathion 83% EC) and 2) CT mass trapping system (240 ml liquid bait in its trap). Traps were at the southern part of the tree at 1.5 – 2 from the ground and no refills during the field trial were done.

## Results and Discussion

### *Electrophysiological response*

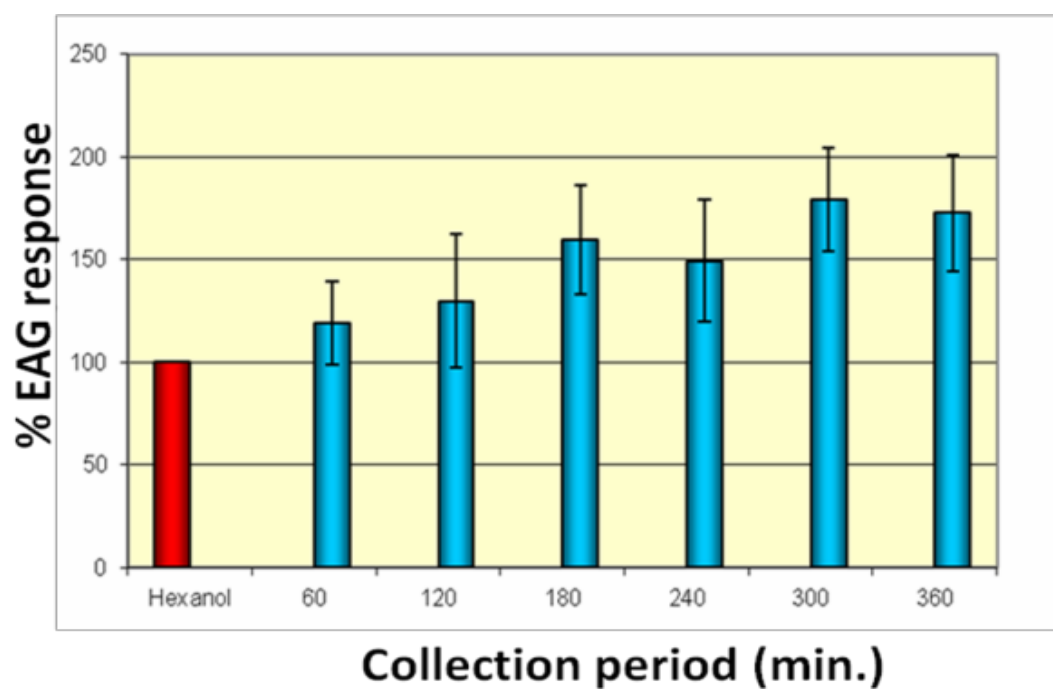
EAG dose response revealed that over the range of concentration evaluated, the EAG response to the volatiles released by Cera Trap was higher for females (Fig. 1) than for male response (Fig. 2) at any collection time doses tested.

Although slight differences between males and females in the EAG response were found, only in few cases the differences were significant. EAG recordings reveal a *C. capitata* dose – dependent response to the volatiles released by the lure.

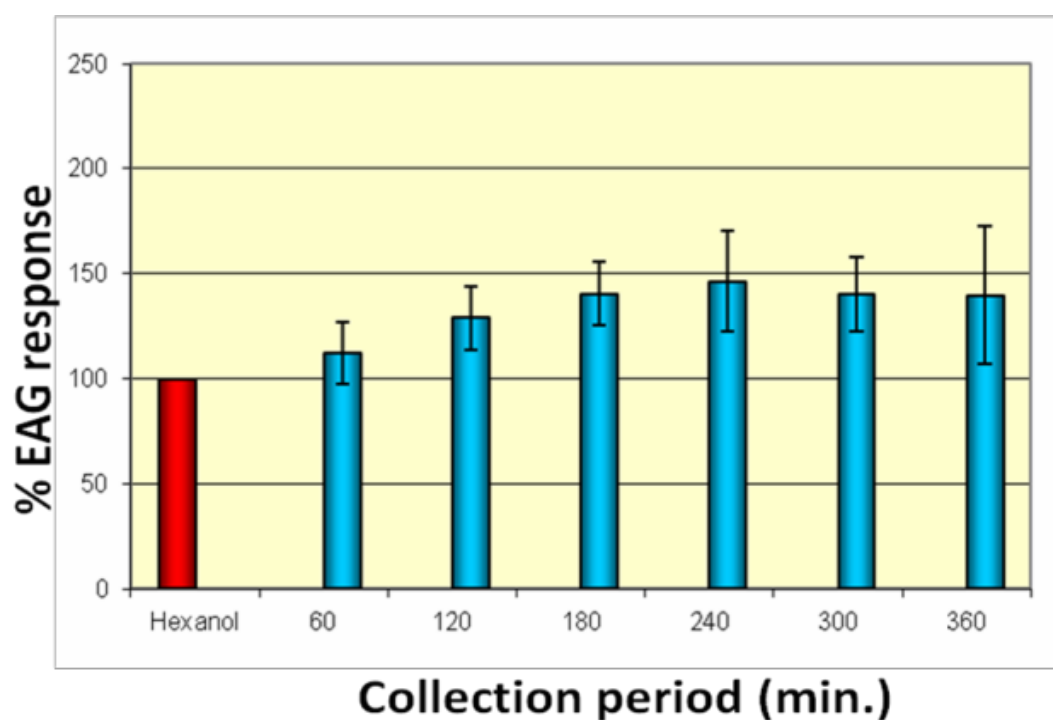
With a constant dose of 1 µl containing EE for a 180 min collection period, the female response varied with its age. The maximum EAG response, 154% ± 55, was obtained from immature 4 days-old females (Fig. 3).

### *Field trial*

Total numbers of males and female flies captured per trap by the two different systems evaluated are given in Fig.4 and 5.



**Fig. 1.** EAG response (%) (Mean  $\pm$  SD) of *C. capitata* (n=5) females to 1  $\mu$ l dose of Cera Trap at different collection periods (min.).



**Fig. 2.** EAG response (%) (Mean  $\pm$  SD) of *C. capitata* (n=5) males to 1  $\mu$ l dose of Cera Trap at different collection periods (min.).

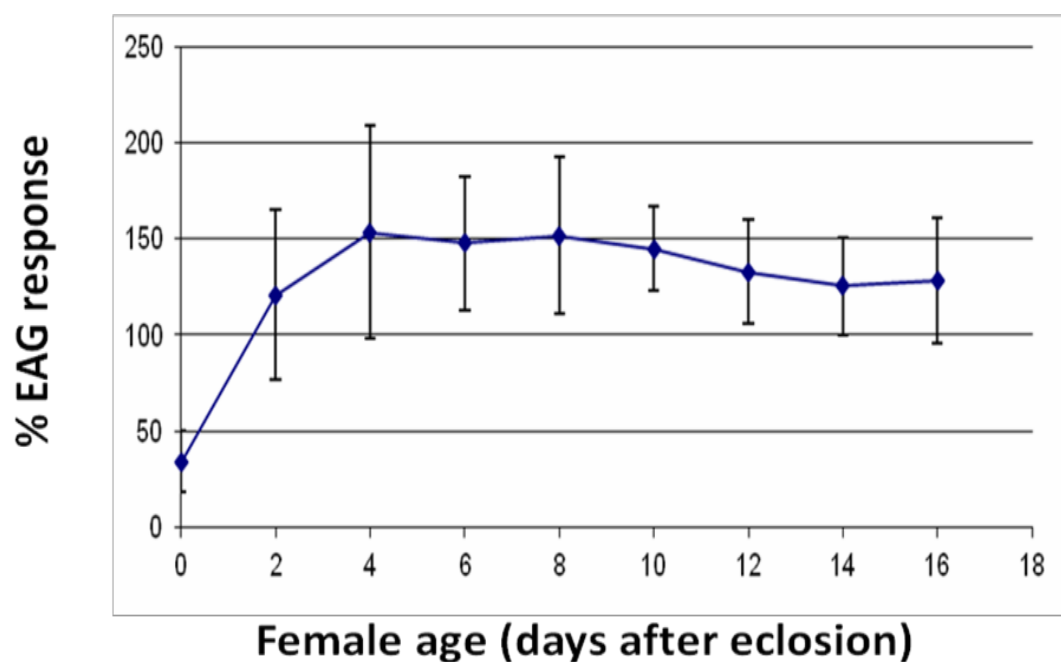


Fig. 3. EAG response (%) (Mean  $\pm$  SD) of *C. capitata* females (n = 5) vs. age.

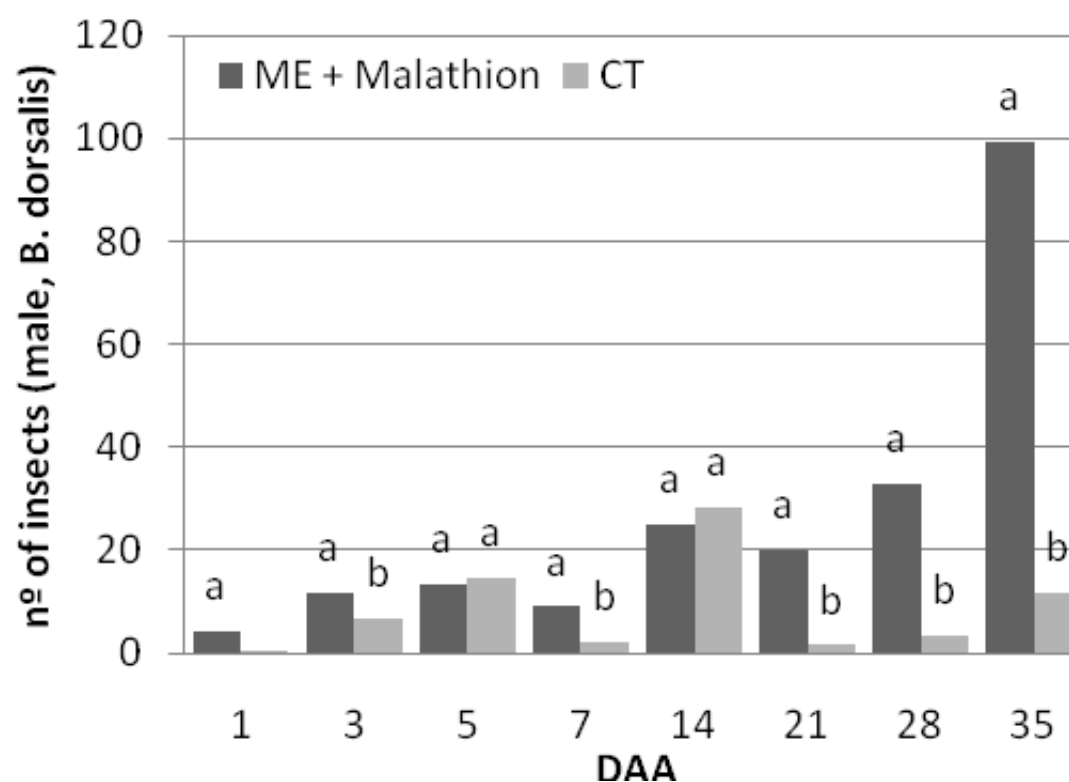
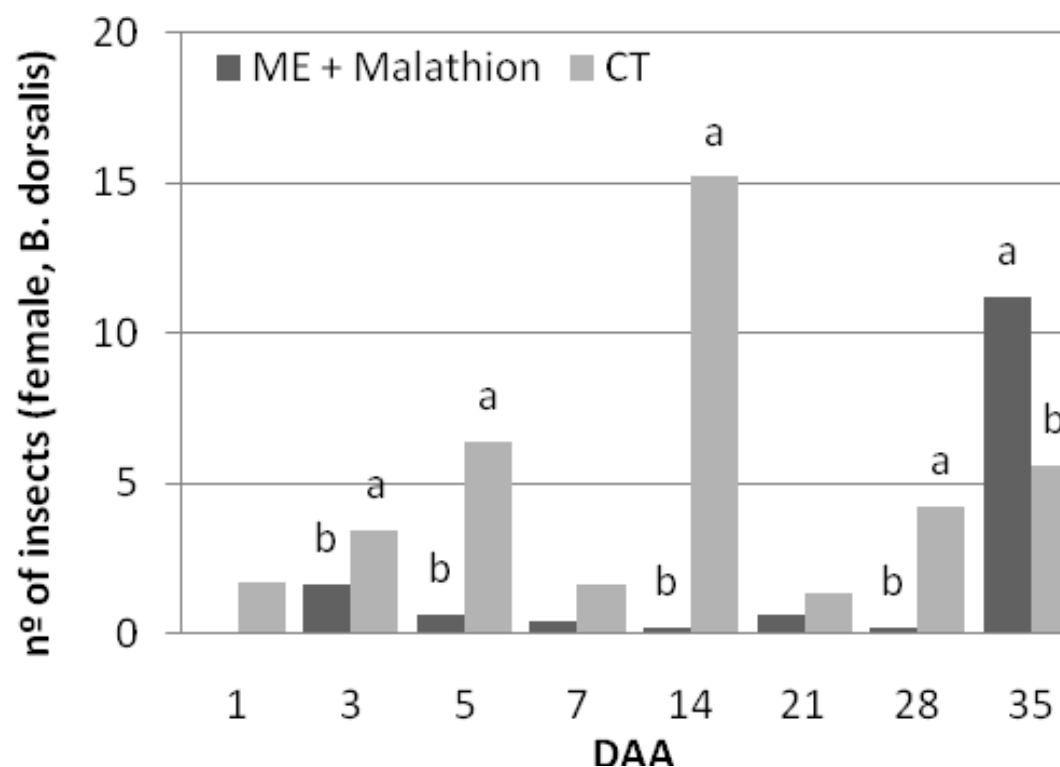


Fig. 4. Number of males captured (*B. dorsalis*) at 1-35 days after traps placement. Different letters represent statistically significant difference at 95% level by Duncan's multiple range test.



**Fig. 5.** Number of females captured (*B. dorsalis*) at 1-35 days after traps placement. Different letters represent statistically significant difference at 95% level by Duncan's multiple range test.

For *B. dorsalis* males, the standard farmer system had a higher level of captures than the CT mass trapping system. In contrast, in females, CT mass trapping system showed higher captures in comparison with the standard farmer system (with statistically significant differences). Therefore, the proposed system could help to avoid female ovipositions that are a key success factor to control this important mango pest before its outbreak.

In conclusion, the compounds released by Cera Trap are detected by the antennae of *C. capitata*. Although the use of higher collection periods of released volatiles from a constant volume does not increase the relative EAG signal.

Regarding differences between sexes, over the evaluated range of concentration, EAG female response is greater than male response at all collection time doses tested though without significant differences.

Perhaps more interesting is the dependencies of the EAG response with the female age. The maximum EAG activity to the Cera Trap released compounds is reached for the immature females (4 days), a very important fact in terms of breaking the pest reproduction cycle.

Finally, field trial results show that the mass trapping technique using Cera Trap liquid bait lure provides a high level of captures of *B. dorsalis*, particularly females, and therefore it could be considered as a useful tool for Integrated Pest Management strategies.

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# **Biology, Ecology, Physiology & Behaviour**

## **A review on the Tephritid fruit flies of economic interest in Cuba: species, plant hosts, surveillance methods and management program implementation**

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### **Abstract**

**Background:** The presence of several Tephritid species in Cuba required of special surveillance methods to determine the free-pest zone or at least to determine the exact species inhabiting the island and the economical repercussion that could affect to the export market. Our previous studies of surveillance, monitoring and training methods set up the protocols for an area-wide fruit fly management irrespectively of the fruit species. In this work, we upgraded the surveillance of *Anastrepha* species in export commodities and in other crops.

**Methods:** Several commodities (fruit varieties) were sampled including not host fruits. A deep surveillance of citrus was also included. Collected infested fruits were retrieved to the laboratory to allow larva development to identify emerged adults to species level. Monitoring traps were also used in citrus plots to confirm the *Anastrepha suspensa*-free status of this commodity.

**Results:** Monitoring traps allowed to confirm the presence of different *Anastrepha* species in Cuba namely *A. suspensa*, *A. soroana*, *A. obliqua*, *A. ocrexia*, *A. insulae* and *A. interrupta*, and also *Toxotrypana curvicauda*. An additional species, belonging to *Anastrepha* genus, *A. sp.*, was also recorded, not matching any described species. Fruit fly major population peak was found to fit with ripening season of stone fruits and other non-citrus fruits. Following the surveillance of tephritid larva' infested fruits, five new host species were confirmed for *Anastrepha suspensa*: sapodilla, rose apple, cocoplum, custard apple, and gac fruit. And two new ones for *Anastrepha obliqua*: the cocoplum and yellow mombin. Citrus surveillance was clear, no tephritid fruit fly was found in any *Citrus* species in all along Cuba Island.

**Conclusions:** The absence of fruit flies in citrus commodities reveals the success of the implemented management program including surveillance, monitoring and personnel training, putting into value the area-wide Cuban fruit fly management program for *Anastrepha* species.

**Keywords:** *Anastrepha* spp., host status, monitoring systems.



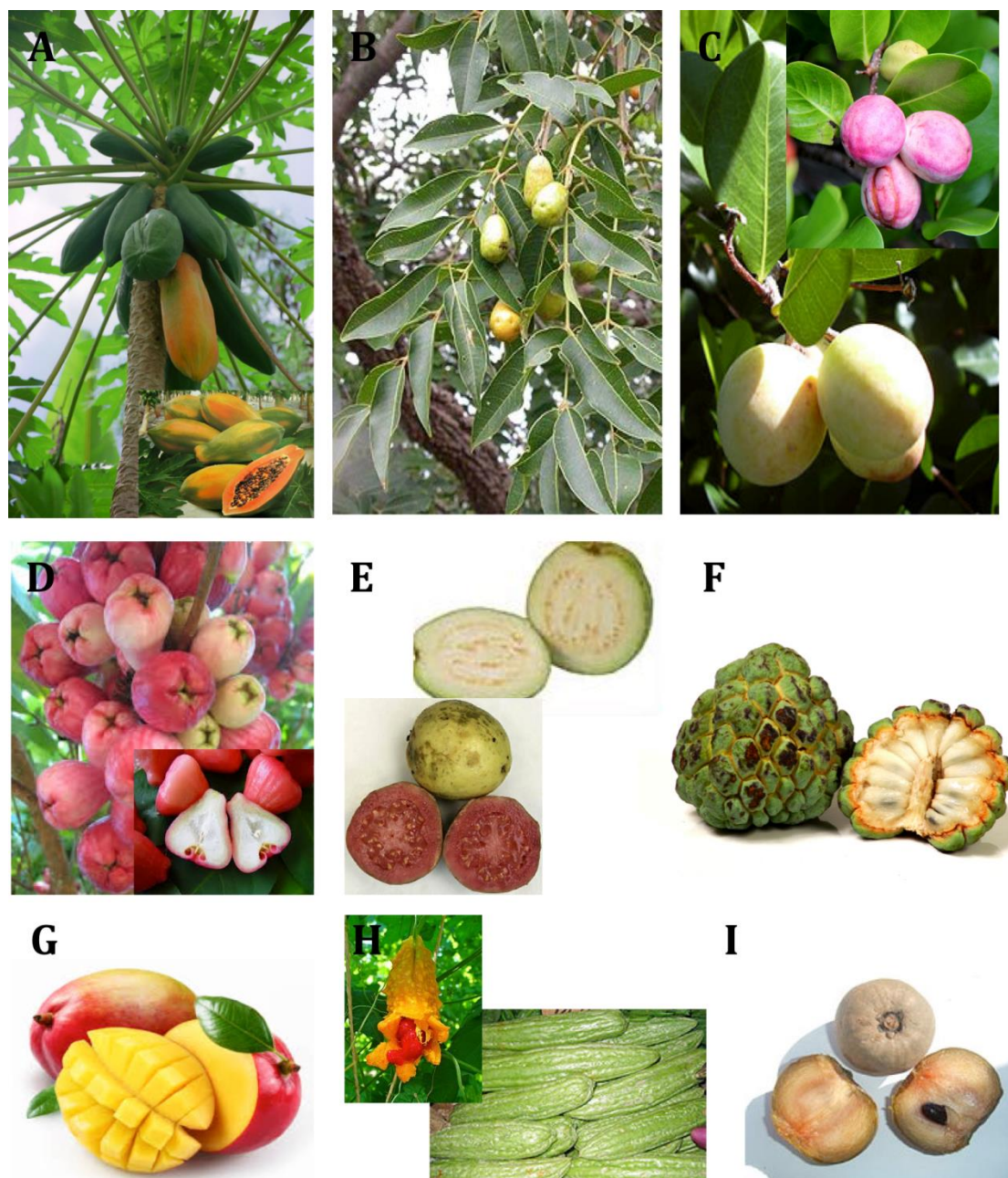
## Introduction

The Cuban citrus industry experienced a decrease of production in the early 90's, comparable with that experienced in the early 60's. However, early in this new century (S. XXI), the production has recovered its yield. With more than 109,000 hectares devoted to fruit cultivation, only 20% are dedicated to citrus whereas more than 24% are devoted to mango groves, which highlighted the Cuba ability to open its fruit market. The Cuban citrus and fruit industry operates within Fruit Trees Company Group (GEF), the Ministry of Agriculture, several small companies and the Tropical Fruit Trees Research Institute (IIFT) that provide scientific and technical support for all Cuban fruit culture (FAO, 2003).

Nowadays, Cuba is working to develop the country's fruit demand in a sustainable way in order to cover both internal demand (population and tourist facilities) and exports markets but not only focused in Citrus (<http://www.atcitrus.com/english/15460>) also covering other fruits trees (Fig. 1). Within this fame, the IIFT had developed special methodologies to control pest insects that would affect this new age of fruit production. More precisely, the Ecology joint unit formed by IIFT and IES (Ecology and Systems Institute), had developed a laboratory for fruit fly species and their hosts identification. These two research units are also responsible of surveillance, monitoring and training methods to set up protocols for an area-wide fruit fly management (Borges-Soto et al., 2011, 2015). These protocols cover one of the key insect pests, the Tephritidae fruit flies. This group of flies encompasses one of the most destructive world-wide distributed pest species, grouped in three main genera, *Anastrepha*, *Bactrocera* and *Ceratitis*, many of them not present in the Cuba Island.

In Cuba it had been identified ~30 species of tephritids, belonging to 15 genera (in alphabetical order: *Acinia*, *Acrotaenia*, *Anastrepha*, *Blephanroneura*, *Dioxyna*, *Dyseuresta*, *Euaresta*, *Euarestoides*, *Hexachaeta*, *Tetreuaresta*, *Tomoplagia*, *Toxotrypana*, *Trupanea* and *Xanthaciura*), with many of them without reference to their host plant (Fernandez et al., 1997; Rodriguez Velásquez et al., 2001; Ovruski et al., 2005). This list of species was obtained mainly from the Zoological Collection repository at the IES, meaning that in most cases the description is based on the type or holotype specimen archived, not from captured material, for which its real status of presence and the estimation of their economic impact on crops production in the Cuba Island still requires further research. The geographical localization of Cuba Island, its climatic situation with increase of strong climatic events (i.e. hurricanes, tropical storms, etc.) and the market opening along the establishment of new cultivars could benefit the arrival and establishment of new species from the surrounding countries. As an example of highly pestiferous Tephritidae species found in the Cuba island' neighbors we can found members of *Bactrocera* and *Ceratitis* genus; *Bactrocera correcta* is regularly trapped in Florida, and even *Ceratitis capitata* which often invades also Florida induce the establishment of SIT and other eradication methods with a great economic impact on citrus growers (Thomas et al., 2010). Thus, the presence of any of these species in Cuba will represent a negative point in their export market, by the quarantine measures imposed by other countries in which species are not present (i.e. USA, Mexico, among others), and a

negative impact in the fruit gross production by the direct damage that these species exerts to the production.



**Fig. 1.** Some of the fruits, other than Citrus, in production in Cuba, that are susceptible of being attacked by Tephritidae fruit flies. A. Papaya, tree and open fruit; B. Jobo, Yellow monbin or Hog plum; C. Icaco or Cocoplum (*Chrysobalanus icaco* Lin.); D. Pomarrosa or Rose apple (*Syzygium jambos* L.); E. Guava or Guayaba; F. Mamón or Custard Apple (*Anona reticulata* L.); G. Mango; H. Cundeamor or Gac fruit (*Momordica cochinchinensis*), one ripe open fruit in the plant and some market size; and I. Níspero or Sapodilla (*Manilkara zapotilla* (Jacq.) Gilly)). Pictures were taken mostly from Wikipedia.

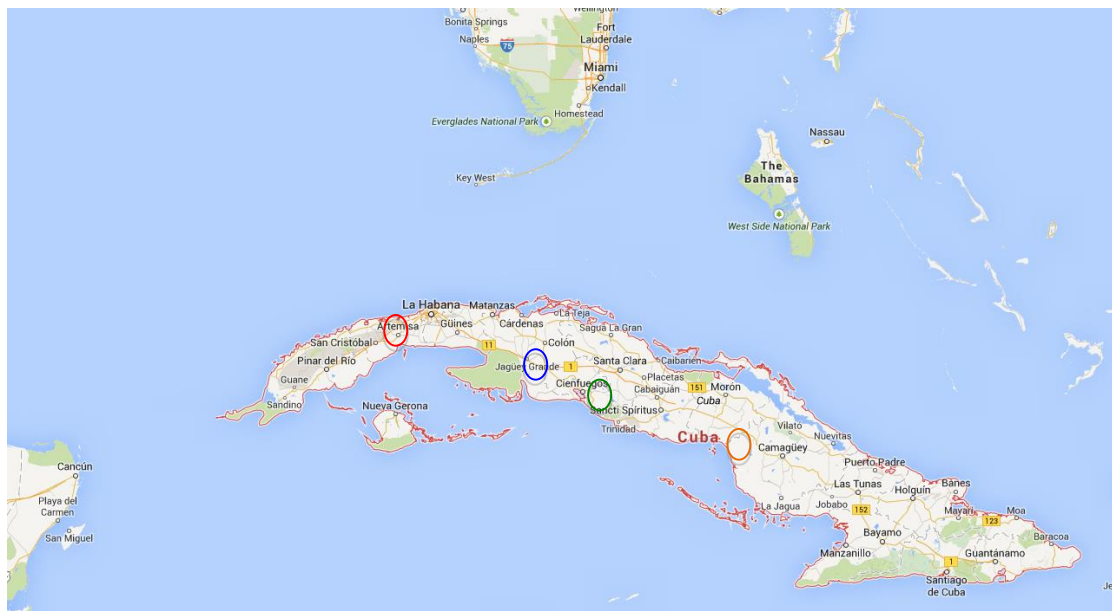
In this context, it was advisable to continue the work developed during the past decade by the Entomology-Ecology unit of IIFT (Borges-Soto et al., 2011). Of special interest were the

developed training methods to be spread among Cooperatives for Agricultural production staff members and growers. But, also, the highlighted and supported the surveillance trap-net around all the citrus production areas, and the monitoring system for other fruit crops (Borges-Soto et al., 2015). It is within this last point on which lies the main objective of the present work: a review on the establishment and pursuing of surveillance methods focused mainly, but not restricted to the *Anastrepha* genus, including the determination of host species sensitive to these fruit flies, and with some review on the detection and identification of natural enemies.

## Material and Methods

### Study sites

Four Citrus companies were selected: Ceiba (Artemisa), Victoria de Girón (Jagüey Grande, Matanzas), Arimao (Cienfuegos), and Ciego de Ávila, located along Cuba island (Fig. 2).



**Fig. 2.** Location of four selected Citrus companies in Cuba: Ceiba (Artemisa) in red; Victoria de Girón (Jagüey Grande, Matanzas) in blue; Arimao (Cienfuegos) in green; and Ciego de Ávila in orange. Picture from Google maps.

The 'Empresa Cítricos Ceiba' (Caimito) has 9.2 ha of production, distributed into 92 plots of unknown area including houses, farms and other buildings, roads and 'no-crop areas' with some wild plants ([https://www.ecured.cu/Empresa\\_C%C3%ADtricos\\_Ceiba\\_\(Caimito\)](https://www.ecured.cu/Empresa_C%C3%ADtricos_Ceiba_(Caimito))). Cultivars include oranges, lemons, grapefruit, guava, mango, papaya (also known as 'fruta bomba') and several vegetables (tomatoes, sweet peppers, cucumbers, among others for local markets and own consumption). Noticeable is one of the selected plots for study in which citrus and guava are alternated (i.e. one row of citrus trees and one row of guava trees).

The 'Empresa Cítricos Jagüey Grande-Victoria de Girón', actually has 25,459 ha for crop production ([https://www.ecured.cu/Jag%C3%BCey\\_Grande\\_\(Jag%C3%BCey\\_Grande\)\)](https://www.ecured.cu/Jag%C3%BCey_Grande_(Jag%C3%BCey_Grande))) of several citrus species, mango, guava, papaya, aguacate and vegetables.

The 'Empresa Citricos Arimao' moved from the ~ 2,000 ha to less than ~1,200 ha (<http://www.opciones.cu/cuba/2013-02-15/empresa-citricos-arimao-una-organizacion-que-fructifica/>). In this enterprise, production includes orange, lemon, grapefruit, mango, guava, papaya and vegetables.

The 'Empresa Ceballos-Ciego de Ávila' with more than 8,000 ha, include orange, lemon, grapefruit, mango, guava, papaya, pineapple, tomatoes and other vegetables, such as potatoes and other roots, and sugarcane as principal crops (<http://www.invasor.cu/economia/7871-abanderada-de-la-eficiencia-empresa-agroindustrial-de-ciego-de-avila>).

All the companies include not only the citrus or other fruits plots, but also houses, farms, the roads, and non-crop areas where some alternative hosts can be found.

At all locations, monitoring systems were set up to determine Tephritidae fruit flies presence, population fluctuation, host fruits preference and natural enemies' presence. Monitoring was performed as described previously (Borges-Soto et al., 2010; 2015), which consisted on trapping and fruit survey as explained below.

#### *Trapping systems*

Three kinds of traps were used: Mc Phail, Rebell and Jackson. Trimedlure (targeting *C. capitata* males) was used to lure some of the traps in all the study plots. Traps contained also food attractants, to target all other Tephritidae species females, either Torula yeast or sugar cane molasses. Different concentrations of food attractants were tested in one of the study sites. Baited traps were located at different points within the Citrus enterprises; other surrounded fruits crops were surveyed too (Table 1). Mc Phail traps were serviced on a weekly basis whereas Rebell and Jackson were serviced on a quarterly basis as determined previously (Borgues-Soto et al., 2011). At each revision time, trap content was transferred to 70% ethanol vials, labeled and retrieved to the IIFT laboratory for species identification. Traps were surveyed from 2010 to 2013.

All *Anastrepha* species and parasitoids were separated and stored in amber glass vials with 70% ethanol for further processing. Specimens were first isolated from the whole trap capture and identified under binocular microscope with the aid of the corresponding taxonomic keys.

Infestation level was determined using FTD formula (Colling Sanchez, 1994):

$FTD = \text{Total no. flies} / (\text{Traps/ha}) * \text{sampling period (in days)}$

#### Category FTD

Null 0.00

Low <0.01

Medium 0.01-0.08

High >0.08

**Table 1.** Monitoring traps used at each Citrus enterprise, fruit culture type or location, total trap number per culture (per hectare), and bait/lure used in each type.

Culture type or location	Trap type	Total <sup>1</sup>	Bait <sup>2</sup>
(E.C.) Ceiba, Artemisa			
Citrus	Rebell	12	Torula yeast
	Jackson		Trimedlure
Mango, Papaya, Guava	Rebell	9	Torula yeast
	Jackson		Trimedlure
	Mc Phail		
(E.C.) Jagüey Grande-Victoria de Girón'			
Citrus	Rebell	nd	Torula yeast
	Jackson		Trimedlure
Mango, Papaya, Guava	Rebell	nd	Torula yeast
	Jackson		Trimedlure
	Mc Phail		
(E.C.) Arimao, Cienfuegos			
Citrus	Mc Phail	3	Torula yeast
			Trimedlure
Mango, Papaya, Guava	Mc Phail	4	Torula yeast
			Trimedlure
(E.C.) Ciego de Ávila			
Citrus	Jackson	3	Trimedlure
Guava	Rebell	2	Trimedlure
	Mc Phail	1	Torula yeast
Plum	Mc Phail	1	Torula yeast
Mango	Mc Phail	1	Torula yeast
	Rebell	1	Trimedlure
Papaya	Jackson	1	Torula yeast
		1	Trimedlure
Postharvest Plant	Rebell	1	Trimedlure
	Mc Phail	1	Torula yeast
Neighborhood	Jackson	1	Trimedlure

<sup>1</sup>Total number of traps, each cover at least one hectare of cultivar; <sup>2</sup>Food baits were either Torula yeast or sugar cane molasses depending on availability; nd, not determined.

### *Fruit collection and inspection system*

In all four Citrus companies, citrus fruits and other deciduous cultivated fruits (mango and guava mainly) were collected before (at color changing) and at ripening (after complete color change) stages. In this study, also non cultivated fruits (alternative hosts) present in the vicinity of the Citrus companies or found between Citrus and other cultivars (mango, guava, papaya) were also collected (ripe fallen fruits were excluded).

Citrus fruits (mainly Navel oranges and grapefruits) were inspected for oviposition scars under binocular in place at pre-ripening and at laboratory at ripening stage (all at pre-harvest time). At

least 10 trees with 10 fruits per tree, per site and sampling date were randomly selected for oviposition scars inspection.

All the fruits with clear oviposition damage or other kind of damage due to insect feeding were labeled and retrieved to the laboratory. At laboratory, fruits were dissected to inspect for Tephritidae larva and natural enemies (like entomopathogenic nematodes) presence. After determination of larva presence, these fruits were stored to allow development into adult for complete species identification, including identification of parasitoids. Emerged adults (fruit fly or parasitoid) were preserved in amber crystal vials with 70% ethanol for further studies.

All fruits were packaged following Cuba's rules 70-11 to avoid fruit fly or other pests spread among Citrus Enterprises.

## Results and Discussion

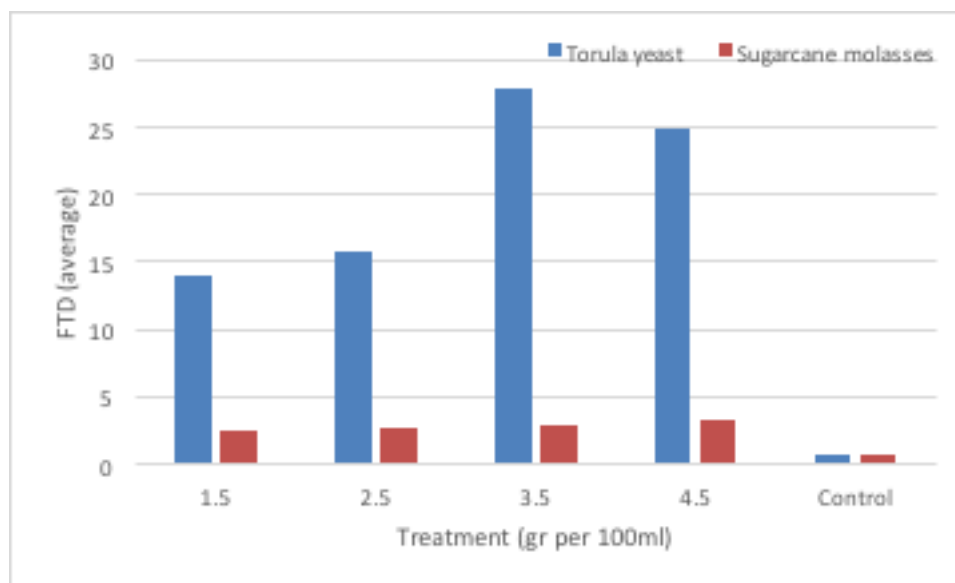
### Trapping

In overall, monitoring traps allowed confirming the presence of different *Anastrepha* species, previously reported to be found in Cuba: *A. suspensa*, *A. soroana*, *A. obliqua*, *A. ocrexia*, *A. insulae* and *A. interrupta*. These traps also recorded an unnamed one, here identified as *A. sp.*, not matching any morphological description in the used keys, nor in the past reports of species lists (Fernandez et al., 1997; Rodríguez Velásquez et al., 2001). Several voucher specimens of this unnamed species *A. sp.* have been deposited at the IES institute collection for further research. Another species was identified as *Toxotrypana curvicauda*, however this species was found only in traps from papaya plots.

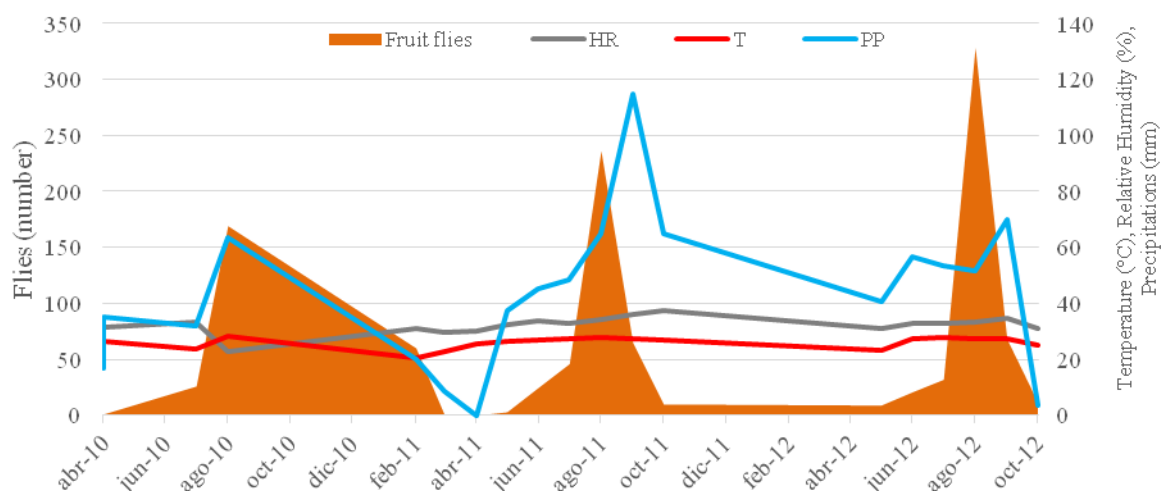
From the results, we observed that from the three types of traps used, Mc Phail was the best in capturing fruit flies, followed by the Rebell type (data not shown). Of the two food attractants used, Torula yeast or sugar cane molasses, the Torula yeast was the best for *Anastrepha* species even at the lowest concentration used (Fig. 3).

Fruit fly major population peak was found to fit with ripening season of stone fruits and other non-citrus fruits, which lies around August month, also fitting the raining season (Fig. 4). Despite this high number at ripening time, overall FTD was of 0.0054 *Anastrepha* sp. (pooled fly species per trap and day along the three seasons tested) indicating a low prevalence of flies in the Citrus enterprises.





**Fig. 3.** Average *Anastrepha suspensa* captures (as FTD) in Mc Phail traps baited with Torula yeast (blue) or with sugarcane molasses (red) at different concentrations. Traps were located in one of the guava plots at 'E.C. Jagüey Grande-Victoria de Girón'. Control traps contained 51 cc of hydrolysate yeast used elsewhere as control bait.



**Fig. 4.** Population dynamics and total captures of *Anastrepha* spp. During three seasons in four sites in Cuba, with relation to environmental conditions (average temperature (Celsius degrees; in red), relative humidity (percentage; in dark grey) and precipitation (average mm raining; in light blue).

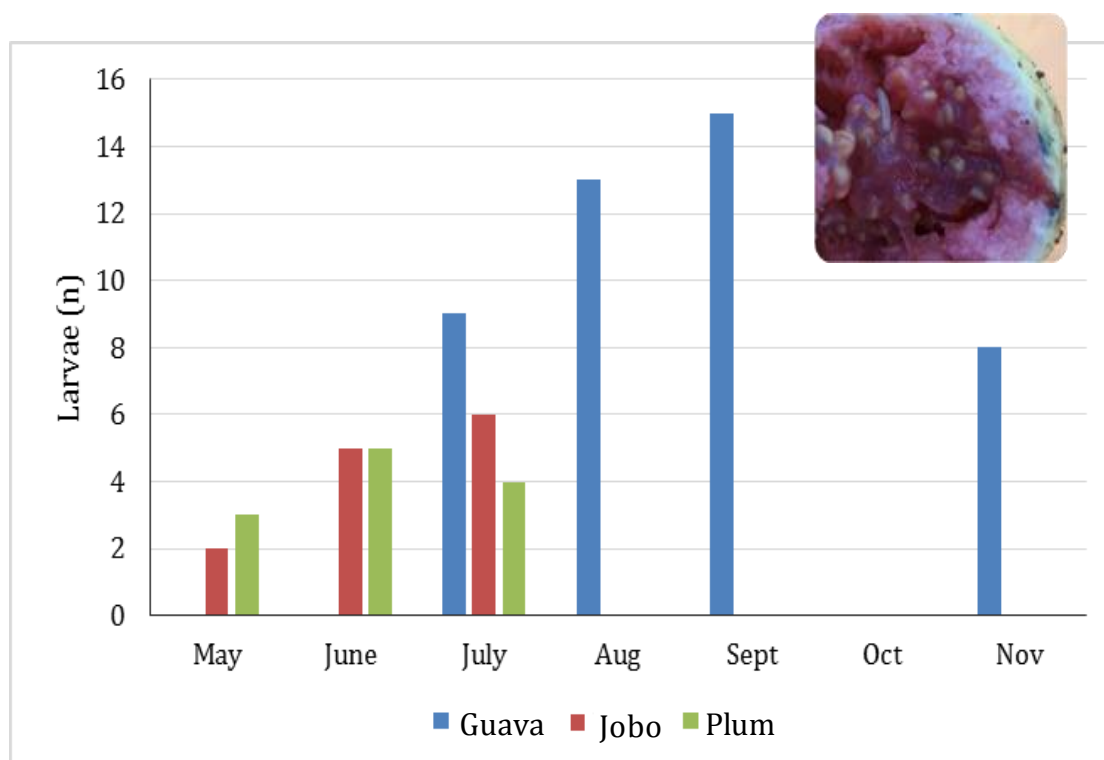
Traps baited with Trimedlure as lure were setup according to a National program for the surveillance of arrival of *C. capitata* to Cuba from neighboring countries mediated by strong climatic events (i.e. hurricanes). None of the traps reported any medfly specimen, as in the previous decade, indicating that the surveyed areas are free from medfly, fulfilling one of the quarantine measures required from citrus importing countries (i.e. USA, Canada). This result can be extended to the whole country, as the study plots covered the most important citrus production areas of the country.

### Fruit surveillance

In the citrus fruits surveillance, Tephritidae fruit flies were obtained from near 1,000 fruits collected in any of the four companies evaluated, despite the species captured in traps in the four locations.

Among the commercial fruits, guava and mango showed the highest larval presence values. Other deciduous or stone fruits collected with oviposition symptoms were retrieved to the laboratory for further processing, as host species determination, fruit fly species identification and natural enemies' detection after complete development of recovered larvae.

As an example, the abundance of *Anastrepha suspensa* larvae in Jagüey Grande followed the ripening status of host fruits. The highest peak for *Spondias mombin* (or Jobo) was on July, for plum in June and for guava in September (Fig. 5).



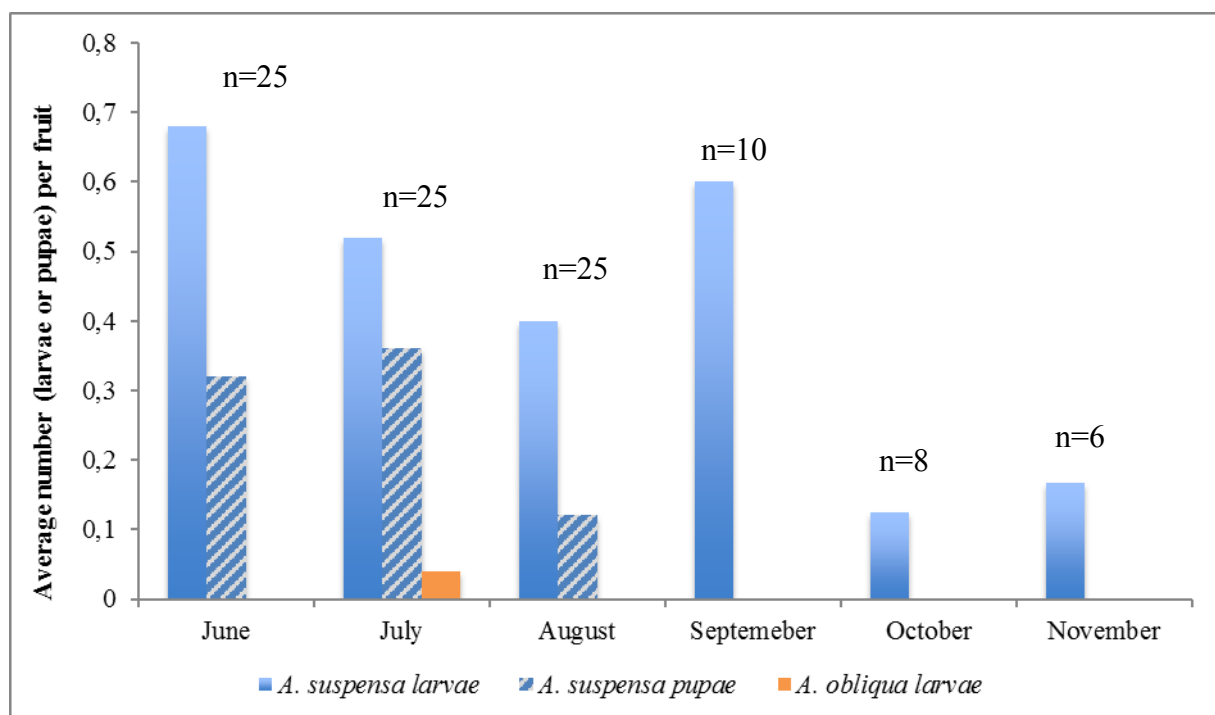
**Fig. 5.** Fluctuation of larvae number (average larvae per fruit) of *Anastrepha suspensa* in several fruits (guava in blue, jobo in red, and plum in green) along the year in Jagüey Grande. A red guava infested fruit is shown to notice the damage produced by the *A. suspensa* larva.

At Ceiba the most affected fruits were guava with a clear population peak around June and another in September (Fig. 6). In this enterprise, the *A. suspensa* population reached approximately 0.68 larvae/fruit at the highest point. However, in this enterprise, *A. obliqua* was detected co-infesting with *A. suspensa* (Fig. 6), but at lower rate (0.04 *A. obliqua* larvae per fruit).







Following the surveillance of Tephritidae larva-infested fruits, five 'new' host species were recorded for *Anastrepha suspensa*: sapodilla (or níspero in Cuban; *Manilkara zapotilla* (Jacq.) Gilly), rose apple (or pomarrosa; *Syzygium jambos* L.), cocoplum (or icaco; *Chrysobalanus icaco* Lin.), custard apple (or mamón; *Anona reticulata* L.), and gac fruit (or cundeamor; *Momordica cochinchinensis* Spreng.). And two new ones for *Anastrepha obliqua*: the cocoplum (Icaco) and yellow mombin or hog plum (jobo fruit in Cuba; *Spondias monbin* L.) (Table 2). Even if this list is considered here new host species, this is due to the consideration of the available literature affecting fruit samples from Cuba.

In previous works were listed the observed Tephritid species and their associated plant hosts, among it appeared *A. suspensa* with sapodilla, rose apple, cocoplum and yellow mombin as host plants (Fernández et al., 1997; Rodríguez Velásquez et al., 2001). But, as stated by the own authors, the distribution of *Anastrepha* species in the Cuba island is unknown and many of the hosts plants assigned to each species is those that appear at the collection data, meaning that the authors didn't performed a deep study on *Anastrepha* species host plants available in Cuba. For this reason, this work summarizes a first step in the identification of commercial and alternative hosts for the economic important *A. suspensa* species.



**Fig. 6.** Average larvae (solid colors) and pupae (striped bars) number per guava fruit along the year in Ceiba (Matanzas, Cuba). The number of fruits collected in each month is indicated above the bars as n.

**Table 2.** Fruit flies detected in evaluation area, with indication of species name, fruit host scientific name and common name. Pictures of the representative fruit fly species type are presented below each name (pictures obtained from <http://paroffit.org>).

Fruit fly species	Fruit host (scientific name)	Fruit host (common name)
<i>Anastrepha suspensa</i> 	<i>Manilkara zapota</i> G *	Níspero or Sapodilla
	<i>Momordica charantia</i> *	Cundeamor or Gac fruit
	<i>Eugenia gamboa</i> L*	Rose apple or Pomarrosa
	<i>Chrysobalanus icaco</i> Lin *	Icaco or Cocoplum
	<i>Mangifera indica</i>	Mango
	<i>Psidium guajava</i> . L	Guayaba or Guava
	<i>Anona reticulata</i> L*	Mamón or Custard Apple
<i>Anastrepha obliqua</i> 	<i>Chrysobalanus icaco</i> Lin*	Icaco or Cocoplum
	<i>Spondias monbin</i> L*	Jobo or Yellow monbin, or Hog plum
	<i>Mangifera indica</i>	Mango
	<i>Chrysophyllum caimito</i> . L	Caimito, Golden leaf tree fruit, star apple
	<i>Spondias monbin</i> L*	Jobo
<hr/>		
<i>Anastrepha ocrexia</i> 	<i>Manilkara zapotilla</i> (Jacq. Gilly)	Níspero or Sapodilla
<hr/>		
<i>Toxotrypana curvicauda</i> 	<i>Carica papaya</i>	Papaya

*Control agents: entomopathogen nematodes and parasitoids*

As it is known, biocontrol agents can be found in agro-ecosystems if a sustainable and environmentally friendly techniques are applied, enhancing and allowing a self-control by spreading into the ecosystem. During the past years, in Citrus enterprises, IPM has been introduced for control tephritid fruit flies and other pests while keeping chemical pesticides at low levels. These low pesticides application had allowed the establishment of biological control of tephritid species. It was found the braconid species *Utetes anastrephae* (Viereck) (Hymenoptera: Braconidae), which seems to contribute greatly to biological control of *Anastrepha* species (Fig. 7). This species has been detected at low numbers (n=12 in 2011; n=11 in 2012; and n= 12 in 2013) in mango and Guava cultivars infested by *Anastrepha suspensa* and/or *A. obliqua*; its large developmental time didn't allow an exact assignment of host.

*Utetes anastrephae* has a wide distribution in temperate areas of continental America ([http://entnemdept.ufl.edu/creatures/beneficial/wasps/utetes\\_anastrephae.htm](http://entnemdept.ufl.edu/creatures/beneficial/wasps/utetes_anastrephae.htm)). This species is thought to be a complex of closely related species with color-variable body, small subtle differences in ovipositor, thorax drawings or host species preferences that deserve further studies (Wharton & Yoder, 2014). This species has been cited exerting different percentages of parasitism in other countries, reaching a maximum parasitism rate of 66.7% against *A. obliqua* when infesting *Spondias mombin* (López et al., 1999), where it was considered a native parasitoid (Silva et al., 2010; Ovruski & Schliserman, 2012; Garcia & Ricalde, 2013). López et al., (1999) also found *U. anastrephae* in guava fruits infested by *Anastrepha* species at 0.1% parasitism rate, whereas these authors did not found any in infested mangoes.

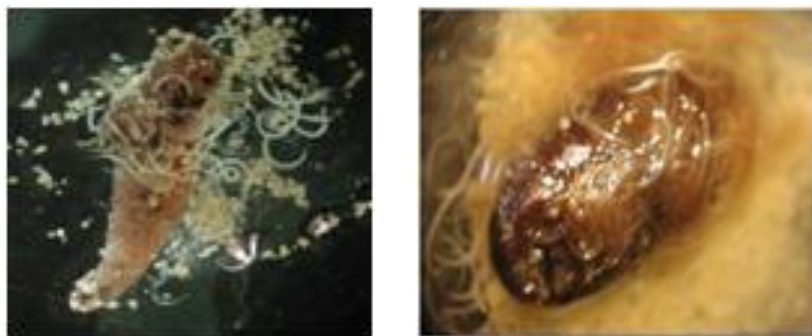
Due to the low detection numbers, we don't know if the species found in this work is native of Cuba or has reach it as specimens travelling with hurricanes or tropical storms. Further research with the aid of molecular markers would be necessary to determine this last point as some morphological variations have been detected, along by continuing the surveillance in infested fruits.



**Fig. 7.** *Utetes anastrephae* (Viereck) specimens in dorsal (left) and lateral (right) view.

In addition to this parasitoid, an entomopathogenic nematode, *Heterorhabditis indica*, has also been found infesting *Anastrepha suspensa* larvae (Fig.8), as also previously reported

(Borgues-Soto et al., 2011). Some assays with this nematode showed a control of *Anastrepha suspensa* larvae by inducing 76.7-86.6% larva mortality, but further research should be done to establish a rearing colony of this nematode, along with the determination of best suited test protocols and field release. This mortality rate is similar to those obtained in laboratory with other entomopathogens like the fungus *Metarhizium anisopliae* (Quesada-Moraga et al., 2008; Dimbi et al., 2013) or *Beauveria bassiana* (Dimbi et al., 2003).



**Fig. 8.** *Heterorhabditis indica* specimens in larva (left) and pupae (right) of *Anastrepha suspensa* (picture from M. Gomez, M. Montes, M. Borges, D. Hernandez and J.L. Rodriguez; 2013).

As explained before, biological control is an environmental friendly technique that seems enhanced by the actual procedure for the Tephritidae fruit flies control program in Cuba. However, this point deserves further research to improve the presence of parasitoids and other natural enemies in Citrus agro-ecosystems with inter-cropping systems.

## Conclusion

The key result of this work is the determination of complete absence of *Ceratitis capitata* and other *Anastrepha* species infesting Cuban citrus fruits, despite the registered captures of *Anastrepha* spp. Adults in baited traps. None of the collected citrus fruits presented oviposition scars nor larva tunnels, even if in their vicinity other plant host species (like icaco, jobo, cundeamor, pomarrosa or níspero) and inter-cropping systems (mango, guava or plum) exists and presents infestation mainly by *Anastrepha* species.

These results highlight the success of the implemented management program in citrus which include surveillance, monitoring and personnel training, putting into value the area-wide fruit fly management program in Cuba for the remaining fruit species. Moreover, it seems that the inter-cropping system developed by the Citrus enterprises, along the presence of alternative *Anastrepha* spp. plant hosts serves as push-pull system protecting Citrus species (Cook et al., 2007; Aluja & Rull, 2009), system that deserves further research to enhance the presence of natural enemies.

## Acknowledgments

The authors would like to acknowledge all the growers in all the four Citrus companies for allowing fruit removal and surveillance. Also, we would like to acknowledge the anonymous reviewers of this work for their help in improving this manuscript.

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## Monitoring data and control ideas for *Drosophila suzukii* in Germany

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### Abstract

The Spotted Wing *Drosophila*, *Drosophila suzukii*, is an emerging agricultural pest of soft-skinned fruits. Since the 1980s, but particularly during the last decade, the small fly has invaded a large number of states in North America and many countries in Europe and South America. Here we present monitoring data from 2012 to 2014 from the region of Southern Hesse in Germany. Within only two years the *Drosophila suzukii* population has grown fast, starting to cause considerable damage to different fruits in 2013 and especially 2014. We discuss possibilities for the application of the sterile insect technique for *Drosophila suzukii* control, including the use of transgenic methods to improve the efficiency of SIT programs.

**Keywords:** spotted wing drosophila, sterile insect technique, transgenesis.

### Introduction

Controlling important agricultural insect pests and the spread of invasive species is a great challenge for a globalized trade of crops. Introduced species can pose a threat to human health, agriculture, livestock and/or the environment. They are a hazard to biodiversity because they can change the environmental composition and displace indigenous species (Pimentel et al., 2000). Indeed, invasive species are one of the main causes for the endangerment of endemic species in the USA, second only to habitat loss (Wilcove et al., 1998). Insects play a major role among invasive pest species since their unintended transport happens easily, for instance in contaminated agricultural products. They are also one of the main factors for losses in agricultural income and capable of damaging agricultural goods at different time points during production, before harvest and during storage (Oerke, 2006). The economic damage caused by insects involves direct crop loss, costs for insecticides and other insect control strategies, and the costs which arise from side effects, like medical treatment for insecticide-poisoned workers (Oliveira et al., 2014). Estimating the total costs for insect control is challenging. One of the most recent studies trying to incorporate as many factors as possible has been done on major agricultural crops in Brazil, where the estimated average of insect-induced annual loss in production is 7.7%, and the economic loss is expected to be over US\$ 17 billion for Brazil (Oliveira et al., 2014).

One of the recent events of insect invasion is the case of the Spotted Wing *Drosophila* (SWD), *Drosophila suzukii* (Matsumura). Originally native to eastern Asia, the fly has recently become a worldwide problem. SWD is a *Drosophilid* species possessing a serrated ovipositor, enabling it to penetrate the fruit skin of many soft-skinned fruits of economic importance (Sasaki & Sato, 1995; Hauser et al., 2009). SWD females prefer fresh to rotting fruits (Mitsui et al., 2006) and are attracted to the characteristic color of ripening fruit for oviposition in many host plant species (Lee et al., 2011). One to three days after oviposition, SWD larvae will hatch and feed on the fruit flesh, resulting in unmarketable fruits (Kanzawa, 1939; Walsh et al., 2011; Cini et al., 2012). The damage induced through SWD is hereby mostly due to the feeding larvae. However, oviposition poses a problem, since the penetration of the fruit skin makes the fruit susceptible for infestations by other *Drosophilids*, fungi, bacteria, or yeast (De Camargo & Phaff, 1957; Molina et al., 1974; Louis et al., 1996; Walsh et al., 2011). Due to the recent and ongoing invasion of the SWD, estimations on economic impact through the SWD are so far only available for few countries. Depending on the locations and the affected crop, revenue losses through control programs and crop damage can be substantial and were estimated to be over US\$ 500 million for California, Oregon and Washington in 2008 (Bolda et al., 2010; Goodhue et al., 2011; Cini et al., 2012).

The SWD was first identified in California and in Spain in 2008 (Calabria et al., 2004; Hauser et al., 2009; Hauser, 2011). Since then, the fly has been detected in many regions in the US and Canada, and has spread around continental Europe and the UK according to information from the European and Mediterranean Plant Protection Organization (EPPO, 2014) and scientific reports (Calabria et al., 2004; Hauser, 2011; Walsh et al., 2011). A population genetic approach suggests that the invasion of the continental United States and Europe were independent events, but tracing back the explicit dispersal history of the SWD is not solved yet (Adrión et al., 2014). SWD has also been reported from Brazil in 2013 (Deprá et al., 2014). The flies can survive and propagate in a high temperature range, and are active both during the hot and dry Spanish and Californian summers and in much colder alpine regions in Japan and Europe. Temperature optimum is thought to be between 20 and 25°C, but reproduction takes place in temperatures up to 30-32°C (Kanzawa, 1939; Walsh et al., 2011; Cini et al., 2012). The fly harbors an enormous reproductive potential. Females lay an average of over 380 eggs during their entire lifespan at a rate of 7-13 eggs per day (Kanzawa, 1939; Calabria et al., 2004). The females are probably the overwintering individuals and will be the founders of the next years population (Kanzawa, 1939; Mitsui et al., 2010; Walsh et al., 2011). Questions regarding winter resorts as well as ethological and physiological properties of overwintering individuals remain open and might play a key role in planning and applying control programs for this emerging worldwide pest.

The SWD shows sensitivity to some commonly used insecticides and a certain amount of crop protection can be attained by this conventional pest control measure (Bruck et al., 2011; Van Timmeren & Isaacs, 2013). However, many insecticides are at least partially washed away by rain or are sensitive to UV light, and almost all require a waiting time of several days up to a



few weeks before harvest (BVL, 2014). This makes insecticide use dependent on the weather conditions and usually offers time windows where the fruits are not sufficiently protected against egg deposition and larval feeding. Rain protection or nets can provide additional protection, but they are expensive and not applicable to all kinds of crop.

The sterile insect technique (SIT) is an insect control strategy that has been developed over half a century ago (Knipling, 1955). The idea behind SIT is to overflow the wild population of an insect species with a large number of sterile males. Eggs deposited after a mating event between a sterile male and a wild female will not develop, consequently resulting in a population decline in the next generation. Since SIT is species-specific, it is an environmentally friendly system for insect pest management. It can be applied area-wide and can reach regions of the landscape, which are inaccessible for other insect control systems like insecticides. Recurring release of sterile males as part of an integrated pest management program can result in the suppression or complete eradication of pest insect populations from certain areas (Vreysen *et al.*, 2000; Wyss, 2000; Hendrichs *et al.*, 2002).

Here we present data on the population dynamics of SWD in the state of Hesse in central Germany for a three years period. We also show some information on fruit infestation and the efficacy of the use of insecticides. Further, we discuss SIT as a potential pest control strategy against the SWD.

## Materials and Methods

### *SWD monitoring*

The SWD population fluctuation was monitored in Southern Hesse under the coordination of the plant protection service of the state of Hesse (Regierungspräsidium Gießen, Dezernat 51.4 – Pflanzenschutzdienst) from 2012 to 2014 involving 19 locations with fruit plantations. Coordinates ranged between 50.86° to 49.72°N and 7.96° and 8.91°E. Flies were trapped in covered 0.5 L plastic cups with holes of approximately 2.5 mm diameter, filled with an attractant solution composed of 35% water, 35% apple vinegar and 30% red wine and a drop of detergent. As the main focus of the monitoring was early warning for local farmers, it was thus not carried out identically at each location and the numbers of traps varied between one and six per location. Within each location, however, the number of traps and the trapping effort was consistent between the years.

### *Fruit sampling*

Fruit natural infestation levels in the field were determined by fruit sampling at a raspberry plantation in Hofheim. Raspberry harvest started during calendar week 26, the first sample was collected at the beginning of week 27 and harvest ended on week 32. Several samples of 100 raspberries were collected and the number of *Drosophila* larvae per fruit sample was evaluated using the salt water technique (Van Timmeren & Isaacs, 2013). *Drosophila* larvae floating on the surface were counted and assumed to be SWD given that although it is not

possible to morphologically discriminate among larvae from different *Drosophila* species there are no native Drosophilid that lay eggs in undamaged fruit and no other exotic species have been reported for the study region.

#### *Impact of chemical control on SWD infestation levels*

In order to determine the impact of chemical control on SWD infestation levels in cherry plantations in Ockstadt, cherries were treated with Mospilan SG and Spinosad (SpinTor 480 SC) 14 and 7 days before harvest according to German plant protection policies. Concentrations were 0.072 kg/ha and per meter canopy height for Spinosad and 0.125 kg/ha and per meter canopy height for Mospilan SG. Several samples of 100 cherries were collected and processed as the raspberries. Sampling started in the calendar week 23 and continued until week 30. Three samples of 100 fruits each collected in week 28 from a non-treated plot served as control. Statistical evaluation consisted of a one-tailed student's t-test.

#### *Weather data*

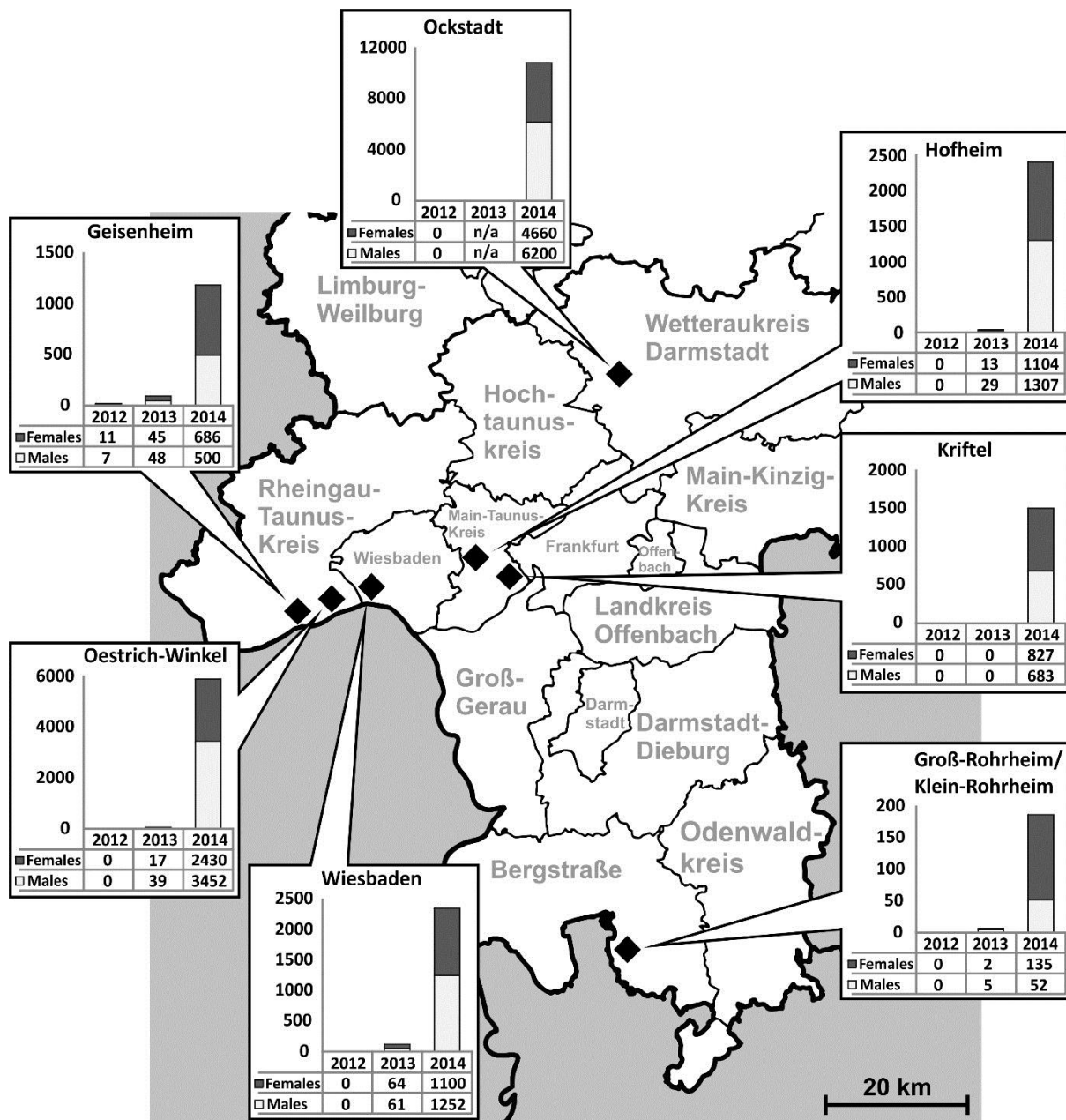
Data for temperature, relative humidity, and precipitation were taken from the open access Climate Data Center of the German Meteorological Service (Deutscher Wetterdienst) (<ftp://ftp-cdc.dwd.de/pub/CDC/>). For the different locations we used the data from the nearest available meteorological stations. These were Geisenheim (station ID 1580, lat. 49.9859 °N, 7.9549 °E, for Oestrich-Winkel), Bad Nauheim (station ID 3442, 50.3574 °N, 8.7506 °E, for Ockstadt) and Wiesbaden-Auringen (station ID 5541, 50.1321 °N, 8.3169 °E, for Wiesbaden). Weekly mean values were calculated for the results section.

## **Results**

For some locations, fly counts were available only for the period up to September 2014. The number of trapped adult flies per year increased dramatically during the monitoring period (Fig.1). While in 2012 a total of 18 adult flies were caught in the field traps, the number increased to 824 in 2013 and reached 24,290 adult flies in the period from January to September in 2014. Moreover, in 2012, SWD was found only at a single location (Geisenheim), while in the subsequent years, flies were found at 16 of 17 and 11 of 12 locations in 2013 and 2014, respectively (Fig.1). In 2014 the absolute number of trapped flies was about 29-fold higher compared to 2013. The highest number of flies was caught in the locations Ockstadt and Oestrich-Winkel, areas of cherry production.

Population dynamics showed similar characteristics for three chosen locations (Fig.2). In spring and early summer only very few individuals were caught, demonstrating a supposedly low population size. In mid to late summer, end of July, a strong increase in the number of trapped flies was detected at all locations. In Ockstadt and Oestrich-Winkel this increase peaked in the last week of August. Afterwards, a rapid decline occurred, corresponding to the end of the cherry harvest season. In Wiesbaden, the population peak at the end of September, beginning of October and the decline occurred at the end of October, five to six weeks later

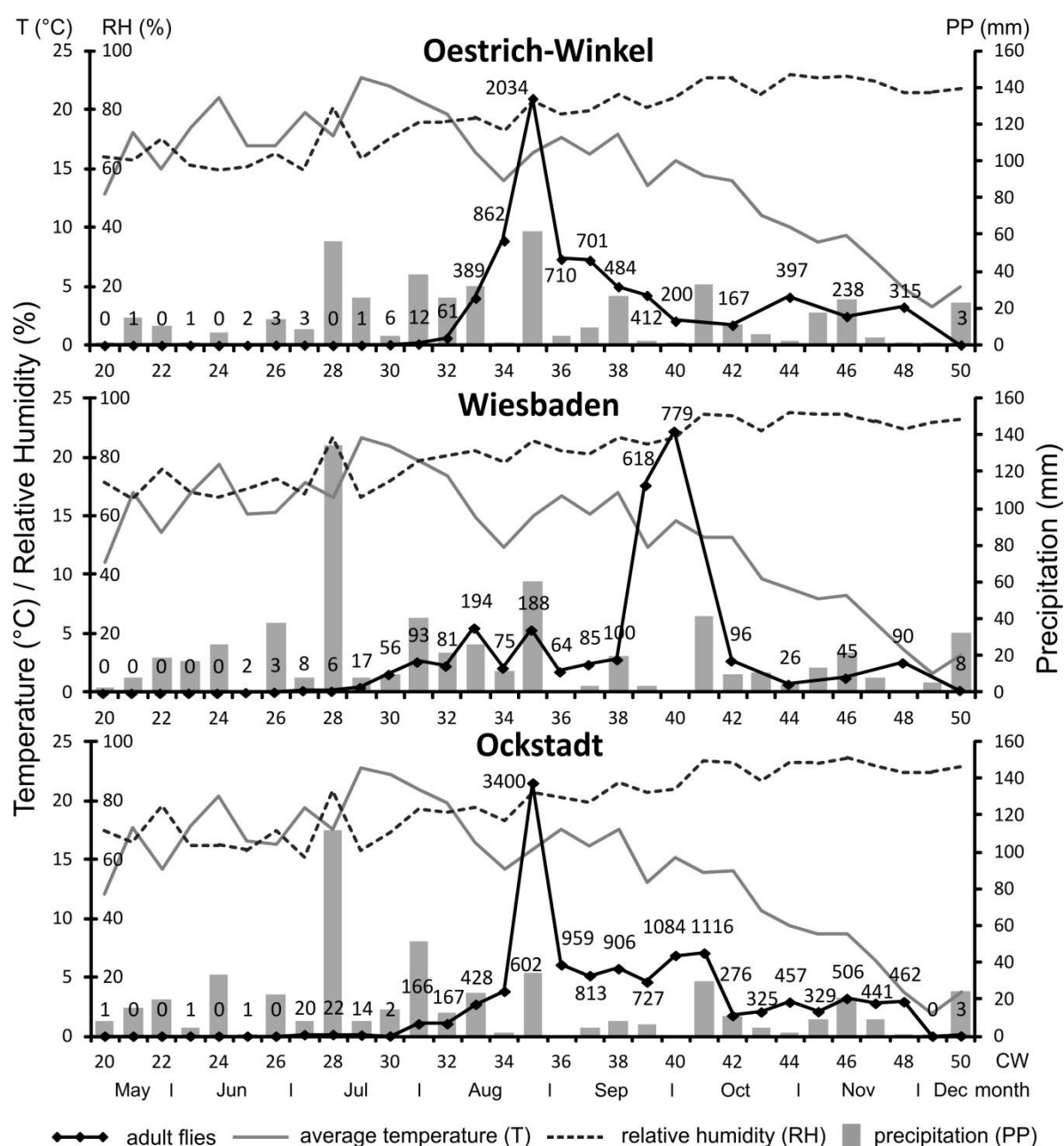
than in Ockstadt and Oestrich-Winkel. Monitoring at all locations was pursued until December and the number of fly catches remained low, although higher than before the harvest.



**Fig. 1.** Monitoring of *D. suzukii* at different locations in southern Hesse from 2012 to 2014. The map shows the position of seven selected monitoring locations and the corresponding numbers of adult flies caught per year, segregated by sex.

Fruit infestation is presented in Table 1. For raspberry, the first sample (week 27) was still free of larvae and the first 4 individuals were detected in week 28. In week 29, 55 larvae were counted, which corresponded to an infestation rate of 0.55 larvae per analyzed raspberry. Until the end of the harvest period, the infestation rate remained at a level between 0.64 and

0.80 larvae per fruit. No SWD larvae were found in the insecticide-treated cherry samples for weeks 23 to 28. However, 127 SWD larvae were found in the non-insecticide treated sample taken in week 28, which relates to an infestation rate of 0.42 larvae per fruit showing significant differences between treated and non-treated fruit ( $P < 0.001$ ). At the end of the harvest season, SWD larvae were also detected in insecticide-treated cherries. However, the infestation rate was considerably lower than in the non-treated sample and reached only 0.011 and 0.024 larvae in week 29 and 30, respectively. The cherry harvest ended after week 30.



**Fig. 2.** Climate data and population dynamics for *Drosophila suzukii* at three locations in Southern Hesse in 2014. Numbers above the solid black line correspond to weekly catches of adult flies. The Fig. shows the total weekly precipitation, and mean values for temperature and relative humidity for each calendar week (CW).

**Table 1.** Infestation of raspberries and cherries with *Drosophila* larvae.

Month	Calendar week	Raspberries			Cherries			insecticides
		fruits	larvae	larvae/fruit	fruits	larvae	larvae/fruit	
June	23	--	--	--	200	0	0	yes
	24	--	--	--	800	0	0	yes
	25	--	--	--	1200	0	0	yes
	26	--	--	--	1000	0	0	yes
July	27	100	0	0	700	0	0	yes
	28	100	4	0.04	1000	0	0	yes
					300	127	0.42	no*
	29	100	55	0.55	1000	11	0.011	yes
August	30	100	64	0.64	900	22	0.024	yes
	31	100	83	0.83	--	--	--	--
	32	100	70	0.70	--	--	--	--

\* Infestation from a non-treated sample taken at week 28.

## Discussion

### *Monitoring data for SWD in southern Hesse*

The SWD has spread within one year over the entire monitored area, which confirms the high migratory potential described for the fly (Hauser, 2011). Although the data must be interpreted cautiously, since numbers from different locations are not entirely comparable, our data indicate that the fly has first spread over the entire monitored area around southern Hesse in 2013 before the populations grew strongly at almost all places in 2014. The highest fly numbers were counted in Ockstadt, which could be related to the high number of cherry trees in this region, which offer perfect breeding grounds. However, more data is necessary in order to make a clear conclusion about the environmental conditions which lead to the intense population build up of SWD observed between different years. For a better picture of population sizes in different areas, more controlled monitoring strategies using the same traps in a comparable density would be necessary.

### *Population dynamics for SWD in southern Hesse*

The curves for trapped adult flies showed a similar dynamic for all three locations. Few flies were caught during spring and early summer, followed by an exponential growth in mid to late summer. These increases correspond with the availability of food and breeding grounds delivered by ripe fruit in forests and plantations for constant reproduction. There was also a continuous increase in relative humidity during the year and from August on the humidity level remained above 80%. Since *Drosophila suzukii* prefers high humidity, these weather conditions were beneficial for the population (Tochen et al., 2015). We suppose that the increase in numbers of trapped adult flies correlates at least partially to the size of the total local population. However, the attraction of ripening fruits luring the flies from surrounding forests and meadows into the plantations must be considered. More monitoring data, particularly from locations outside of plantations, is needed to understand the migratory

behavior of the flies within the year and in locally defined areas. The population declines after the peak levels observed for calendar week 35 in Ockstadt / Oestrich-Winkel and week 40 in Wiesbaden correspond to the end of the harvesting period and are therefore probably a direct result of reduced host availability and breeding grounds.

#### *Infestation rates and economic impact of SWD invasion in Southern Hesse*

Hesse contributes 2.7% to the total fruit-growing area of Germany. Hesses share in the total cultivated area of tree-fruit, berry-fruits, and strawberries grown in Germany is 1.5%, 4.24%, and 5.2%, respectively, which shows a regional focus on berry and strawberry farming (Breitenfeld, 2013). The data presented here highlights the destructive potential of the SWD and the necessity of pest control strategies. Raspberries were heavily infested with larvae within two to three weeks after onset of the harvest period. Moreover, the observed infestation rate increased by a factor of 13.75 within only one week after the detection of the first larvae. This reveals the necessity of rapid precautions once the first flies and/or larvae are detected as well as the need for early detection methods. Infestation rates in cherries were much lower, which is most likely related to the application of insecticides one and two weeks before harvest as the control sample of untreated cherries was also heavily infested with SWD larvae. Our results are in accordance with data from the Statistical Office of Hesse, which report heavy infestation in raspberries, blackberries, non-insecticide treated cherries, and black elderberries for the year 2013. Moderate infestation rates were reported for gooseberry, highbush blueberry, as well as for black, white, and red currant (HSL, 2014). This shows that quick protection efforts are especially needed for raspberries, blackberries, black elderberries, and all organic fruit produce including cherries.

#### *Chemical control of SWD*

Spinosad and Mospilan SG proved to have a protective effect on cherry. However, these insecticides did not offer a complete protection, since at the end of the harvest season SWD larvae were also detected in insecticide-treated cherries. Moreover, insecticidal pest control also presents several drawbacks. Eradicating local populations of non-indigenous species like SWD solely by chemical control is virtually impossible, since surrounding, non-treated areas, offer a refuge for the fly population. The enormous reproductive potential and the short generation time of SWD present an additional challenge to effective control. Under optimal developmental conditions, SWD larvae will hatch within 24 hours after egg deposition (Kanzawa, 1939; Walsh et al., 2011), enabling the fly to destroy fruit within a weekend. Moreover, organic farming is becoming more and more important and the German government wants to increase the proportion of organically produced crops in Germany to 20% of the utilized agricultural area (BMEL, 2011). Only few substances are allowed for the production of organic crops, which limits the spectrum of potential defense agents against SWD. Finally, chemical treatments have the inherent risk of resistance development, which makes the search for additional, alternative crop protection strategies necessary (Brattsten et al., 1986; Hemingway & Ranson, 2000).

*SIT as potential future control method*

The SIT could offer a solution for the potential drawbacks of chemical control measures. Since SIT is species-specific, SIT programs can be applied on large areas without the risk to endanger native species. Because sterile insects can spread and search for potential mates, a systematic, recurring release of sterile insects can in some cases result in the eradication of local populations (Vreysen *et al.*, 2000; Wyss, 2000). Our observed population dynamics for SWD show a low population for spring and early summer and strong and fast increases during mid to late summer. This might be characteristic for SWD populations in temperate climates. For effective SIT programs a large excess of sterile males with respect to the wild-type population needs to be released (Rhode *et al.*, 1971; Dyck *et al.*, 2005). While the high numbers of flies in late summer most likely prohibit the effective application of SIT, the small populations early in the year might be sufficiently susceptible. Large field monitoring studies would be necessary to get a better picture of the SWD population throughout the year and to assess the necessary dimension of an SIT project. Technical aspects concerning mass rearing and infrastructure need to be developed and an efficient sterilization protocol for SWD has to be established. One important aspect for successful SIT programs is the availability of sexing strains. Male-only releases do not only result in a much higher efficiency of SIT projects (Rendon *et al.*, 2000; Cáceres *et al.*, 2004), but in addition the release of sterilized females would increase the amount of damage due to fruit skin puncturing and bacterial and fungal infections (De Camargo & Phaff, 1957; Molina *et al.*, 1974; Louis *et al.*, 1996; Walsh *et al.*, 2011). A release of females should therefore be avoided. Furthermore, a genetic sexing system that kills females before the larval stage also reduces rearing costs substantially (Schetelig & Handler, 2012; Ogaugwu *et al.*, 2013). So far, no efficient sex sorting system is available for the SWD. While adult flies can easily be distinguished morphologically, there is no simple way to discriminate the sexes of immature stages, much less to separate them. For the medfly *Ceratitis capitata* the generation of genetic sexing strains (GSS) has improved the SIT system remarkably and availability of such a system would also be desirable for the SWD. However, the direct transfer of GSS strains from one species to the other is not possible and the development of such a system could be long and laborious. Instead, sexing strains could be developed using transgene technologies. Transgenic embryonic sexing strains (TESS) using female specific splicing have been established and evaluated for two different Tephritids and have resulted in 100% male only populations (Schetelig & Handler, 2012; Ogaugwu *et al.*, 2013). The transfer of these systems to other species is possible. At the same time, the use of fluorescent markers for transformation constructs of transgenic strains can be used for marking (Handler, 2002; Wimmer, 2005). Germline transformation using *piggyBac* transposable elements has recently been established (Schetelig & Handler, 2013) and the genome of the SWD is sequenced and available online (Chiu *et al.*, 2013). The necessary tools for the creation of SIT strains using transgenic methods are hence available and should help in generating an effective, and economically efficient SWD pest control system.

## Acknowledgements

This work was supported by the Fraunhofer Attract Internal Program under Grant No. Attract 125-600172 “Environment-friendly pest control for the Spotted Wing *Drosophila* (SWD), *Drosophila suzukii*” to MFS and the Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz Center for Insect Biotechnology & Bioresources of the Hessen State Ministry of Higher Education, Research and the Arts (to MFS). We thank M. Henning, H. Müller and T. Storch from the Landesbetrieb Landwirtschaft Hessen (LLH) for their contribution to the *Drosophila suzukii* monitoring.

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## **Female remating behaviour in pest tephritid fruit flies and its implication for the Sterile Insect Technique**

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### **Abstract**

**Background:** The efficiency of the Sterile Insect Technique (SIT) targeting tephritid fruit flies depends not only on sterile males mating with wild females, but also on their ability to transmit an ejaculate and inhibit female remating.

**Methods:** Here we review female remating in tephritid flies of economic importance, inhibition of female remating by males and focus on the factors that can modulate post-copulatory mating behaviour.

**Results:** Remating by females can vary greatly between fruit fly species, both in mating frequency and time elapsed between matings (sexual refractory period). While some species seem to be monandrous, others vary in their degree of polyandry - ranging from only two matings in their lifetime to 8 matings per day. Remating inhibition can occur through sperm, accessory gland products (AGPs) or copulatory courtship. However, the mechanisms by which males inhibit female mating are still poorly understood.

**Conclusions:** Despite many studies on the sexual behaviour of tephritids, we still know little about the processes occurring during the copula and how the different components of the ejaculate can affect female post-copulatory behaviour. AGPs have been shown to affect mating inhibition in *Ceratitis capitata*, *Bactrocera tryoni* and *Anastrepha fraterculus* but not in *Anastrepha ludens* or *Anastrepha suspensa*. Thus, the effect of male AGPs should not be generalized throughout tephritids. Understanding how AGPs modify female post-copulatory behaviour can be useful in developing alternative control tactics such as the use of antiafrodisiac substances.

**Keywords:** accessory gland products, copulation, polyandry, sperm.

### **Female remating**

Polyandry in insects is common across a wide range of taxa (Ridley, 1988; Arnqvist & Nilsson, 2000; Torres-Vila et al., 2004). The family Tephritidae is no exception; there are many studies in several species documenting female remating. Most studies have focused on the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (reviewed by Mossinson & Yuval,

2003). Within the genus *Bactrocera*, female remating has been studied for at least four species, *Bactrocera cucurbitae* (Coquillett) (Kuba & Ito, 1993; Haq et al., 2013), *Bactrocera tryoni* (Froggatt) (Harmer et al., 2006), *Bactrocera cucumis* (French) and *Bactrocera cacuminata* (Hering) (Song et al., 2007; Chinajariyawong et al., 2009). Within the genus *Rhagoletis*, female remating has been documented in *Rhagoletis zoqui* (Bush) (Aluja et al., 2001; Rull et al., 2012; Tadeo et al., 2013), *Rhagoletis solanophaga* (Hernández-Ortiz & Frías) (J. Rull personal communication), *Rhagoletis cingulata* (Loew) (Tadeo-Hernández, 2011), *Rhagoletis ramosae* (Hernández-Ortiz) (Tadeo-Hernández, 2014), *Rhagoletis turpinia* (Hernández-Ortiz) (Aluja et al., 2001) and *Rhagoletis completa* (Cresson) (Rull et al., 2012; Tadeo et al., 2013). In *Rhagoletis pomonella* (Walsh), Opp & Prokopy (2000) observed that females mated up to eight times in a day.

Within *Anastrepha*, the presence of multiple mating in females has been studied in *Anastrepha suspensa* (Loew) (Sivinski et al., 1988), *Anastrepha obliqua* (Macquart) and *Anastrepha ludens* (Loew) (Pérez-Staples et al., 2008a; Aluja et al., 2009; Abraham et al., 2014; Meza et al., 2014), *Anastrepha fraterculus* (Wiedemann) (De Lima et al., 1994; Abraham et al., 2011a;b; 2013; 2014), *Anastrepha bistrigata* (Bezzi) (Silva et al., 1985) and *Anastrepha serpentina* (Wiedemann) (Landeta-Escamilla et al., 2016); while for *Anastrepha sororcula* (Zucchi) (Silva et al., 1985) and *Anastrepha zuelanie* (Stone) (unpublished data S.A.), rematings are more rare.

Below we outline the mechanisms by which males can inhibit females from remating and some factors that can modulate female post-copulatory behaviour.

### **Male mechanisms to inhibit female receptivity**

Males will not necessarily gain full paternity from mating with a female (Simmons, 2001). Female insects have sperm storage organs such as the spermathecae and the ventral receptacle where sperm mixing or stratification can take place from rival males (Pérez-Staples et al., 2007; Bertin et al., 2010; Collins et al., 2012; Scolari et al., 2014; Thomas et al., 2014). Thus, in terms of fitness it is in a male's best interest to inhibit females from remating with other rival males after they have mated. Male mechanisms to inhibit female remating and to delay the renewal of sexual receptivity have been studied in detail mostly in *Drosophila melanogaster* (Meigen) (Wolfner, 2002) and certain mosquitoes species. In general, males can inhibit female remating through sperm, copulatory courtship or through particular peptides in the products of the male accessory glands (AGPs). During mating, these secretions are transferred to the female together with sperm. In particular a sex peptide has been found in *Drosophila melanogaster* and *C. capitata* that can inhibit female remating (Chapman & Davies, 2004; Davies & Chapman, 2006). However, for tephritids, sperm, AGPs or both can act in succession to inhibit female remating, but there seems to be great differences between species in the importance of either component of the ejaculate in rendering a female sexually unreceptive.

Female sexual receptivity is not necessarily a single event, it can be “turned off” in the short term but then females can regain receptivity. Thus, despite male investment in manipulating female receptivity, such receptivity usually returns after a certain period of time. On the other hand female receptivity can be turned off in the long term if, with a single mating, females do not remate. The sexual refractory period refers to the time between successive copulations and is generally related to "mating quality", i.e., how much sperm and AGPs (or of what quality) are transferred to the female during copulation.

### *Sperm effect*

The effect of sperm on female receptivity can be studied directly counting the amount of sperm in females that show willingness to remate, compared with mated females that do not show willingness to remate. In this experimental design (Mossinson & Yuval, 2003; Harmer et al., 2006), for both *C. capitata* and *B. tryoni*, remating females had significantly lower numbers of sperm stored, compared to non-remating females. In *A. fraterculus*, *A. ludens* and *A. serpentina*, sperm numbers by themselves seem to play no role in female sexual inhibition (Landeta-Escamilla et al., 2016; Abraham et al., 2016).

The role of sperm numbers on female remating can also be studied using multiply mated sterile males, which transfer decreasing numbers of sperm to their mates, after each successive copulation. In contrast to *B. cucurbitae* and *B. tryoni*, fertile *A. obliqua* males do not suffer this decrease in sperm numbers with increasing matings, thus no sperm depletion is apparent (Kuba & Ito, 1993; Radhakrishnan et al., 2009; Pérez-Staples & Aluja, 2006). For the two *Bactrocera* species studied, remating inhibition did not depend on the number of sperm transferred during copulation, since sterile males without sperm were equally successful in inhibiting female remating compared to fertile males (Kuba & Ito, 1993; Radhakrishnan et al., 2009). In *B. tryoni* this lack of an effect of sperm numbers was seen up to 30 days after the initial mating (Radhakrishnan et al., 2009).

While the mechanisms responsible for changes that occur in females after copulation is not fully understood, the "sperm effect" seems to act mechanically on spermathecae receptors (Fritz & Turner, 2002), which could trigger a physiological response in females to elicit the production of hormones. The sperm effect is usually related to quantity (sperm numbers). However, sperm quality (sperm viability or motility) could also be related to sexual inhibition.

### *AGP effects*

Accessory gland products of insect are produced in the male accessory glands and are composed of carbohydrates, lipids, other materials (uric acid, prostaglandin, juvenile hormone), and in a great amount of proteins (from simple peptides to large structural molecules) (Gillot, 2003; Perry et al., 2013). This is why they are called "secretions", "products" or directly "proteins" of male accessory glands.

Some of the effects of AGPs on female postmating behaviour have been studied using direct injections of these secretions into virgin females. Among these effects, the most relevant for

pest tephritids are a decrease in sexual receptivity observed in *B. tryoni* (Radhakrishnan & Taylor, 2007), *A. fraterculus* (Abraham et al., 2012) and *C. capitata* (Jang et al., 1999). On the other hand, there are cases where the injection of AGPs homogenates do not induce refractoriness in females, such in *A. suspensa* (Lentz et al., 2009) and *A. ludens* (Abraham et al., 2014), it is still unclear why there are such marked differences between species. In *B. dorsalis* there is an increase in male accessory gland size after mating, and males can inhibit females on successive copulations (Wei et al., 2015a). However, it remains to be seen if AGPs in this species cause female mating inhibition.

#### *Copulation effect*

Additionally, the *stimulus* of the introduction of the male aedeago could suppress female remating, at least in the short-term. In order to test this hypothesis, Miyatake et al. (1999) used “penis-cut” *C. capitata* males (males with part of the aedeago cut, such that they can copulate but there is no ejaculate transfer), and then evaluated remating of females first mated with penis-cut males, compared with control intact males. There was no female remating inhibition when males were prevented from transferring the ejaculate (sperm and AGPs), thus this demonstrates that in *C. capitata* the stimulus of copulation *per se* did not inhibit female remating. Recently, the same was observed in *A. fraterculus* and *A. ludens* (Abraham et al., 2016). However, we cannot ignore the fact that the tip of the aedeagus, with its many facets, spines and crenellations, may be very important in copulatory courtship, with effects on sperm transfer and storage, as well as subsequent effects on receptivity (Eberhard & Pereira, 1993; 1995; Marchini et al., 2001). Thus, the microsurgery would have totally eliminated any such effect. Nevertheless, this approach demonstrates that the full ejaculate in those species is needed for mating inhibition.

In *C. capitata*, and possibly *A. fraterculus* and *A. ludens* more than one mechanism may be involved, combined together through a synergetic effect. Currently it is postulated that different elements may act at different time scales. Thus, the ejaculate can inhibit receptivity in the short-term, by acting on receptors in sperm storage organs and finally, the secretions of the accessory glands of the male can act in the long-term, to change the response of the female to male courtship signals (Delrio & Cavaloro, 1979; Jang, 1995; Miyatake et al., 1999; Mossinson & Yuval, 2003; Gavriel et al., 2009). This change can include modulation of female olfactory behaviour. For example, in *C. capitata*, injections of the AGPs cause chemoreceptive changes in females, producing a switch from attraction to male pheromone to attraction for host volatiles (Jang, 2002).

#### **Factors affecting female remating**

Several fruit flies of economic importance are controlled all over the world with the Sterile Insect Technique (SIT). Mass-rearing and irradiation are necessary for SIT, and both factors can affect male post-copulatory success. For insects controlled through SIT, it is desirable that females remain monandrous so that there will be no additional matings after wild females mate with sterile males. Since SIT programs require that males survive to reach sexual

maturity in the field and are able to mate and induce a refractory period in wild females, numerous attempts to enhance male sexual competitiveness and accelerate the process of sexual maturation have been made (called post-teneral pre-release treatments, reviewed in Caceres et al., 2007; Pereira et al., 2013). Examples are the incorporation of protein into the adult male diet, the acceleration of sexual maturation with a Juvenile hormone mimic (methoprene) or aromatherapy (e.g., with ginger root oil) (Fig.1).

### *Mass-rearing*

The process of mass-rearing *per se* can influence female remating. *Anastrepha ludens* represents an iconic case where as many as 80% of mass-reared females remate (Abraham et al., 2014; Meza et al., 2014). This effect was not observed in females of other species such as *A. fraterculus* and *B. cucurbitae* (Abraham et al., 2011a; Haq et al., 2013), although in those studies females stemmed from a laboratory mass-rearing colony and not from a mass-rearing facility, as was the case for *A. ludens*.

On the other hand, mass-rearing can influence male ability to modulate female remating. For example, *A. fraterculus* females injected with AGPs of laboratory males were less likely to mate, compared with females injected with AGPs of wild males (Abraham et al., 2012), showing that mass-rearing is not always detrimental. In the same way, laboratory (sterile) males are equally capable in suppressing female receptivity compared to wild males, through a natural copulation (Abraham et al., 2013). Similarly, laboratory-reared males were as efficient as wild males in inhibiting female remating in *B. cucurbitae* (Haq et al., 2013). Likewise in *A. ludens*, mass-reared and wild males were just as efficient in inhibiting either wild or mass-reared females (Abraham et al., 2014).

### *Irradiation*

Sterile *C. capitata* males were less able to inhibit female remating, and females mated with sterile males had the shorter refractory periods compared to females mating with wild males (Vera et al., 2003; Gavriel et al., 2009). In *A. serpentina*, females mated with sterile males had higher remating propensity, compared to females mated with fertile males (Landeta-Escamilla et al., 2016). In *A. fraterculus* females injected with AGPs of sterile males had higher remating compared with females injected with AGPs of fertile male AGPs (Abraham et al., 2012). However, sterile males were as efficient as wild males in inhibiting female remating throughout a natural copulation, thus AGPs are not the only component of the ejaculate responsible of female inhibition in this species (Abraham et al., 2013). Similarly, irradiation had no effect on female remating propensity in *B. cucurbitae* (Haq et al., 2013).

### *Male diet*

Male diet can affect sperm numbers and indirectly female remating (Yuval et al., 2002; 2007). Male diet affects sperm production and female remating in at least *C. capitata*, *B. tryoni*, *B. cucurbitae*, *A. fraterculus* and *A. obliqua* (Blay & Yuval, 1997; Taylor & Yuval, 1999; Yuval et al., 2002; Pérez-Staples et al., 2008a;b; Aluja et al., 2009; Gavriel et al., 2009, Abraham et al., 2011b; Costa et al., 2012; Haq et al., 2014) but not in *R. pomonella* (Hendrichs et al.,



1992). In *A. fraterculus*, for example, wild females mated with sugar-fed males remate more often, remate sooner and stored less sperm than females mated with protein-fed males (Abraham et al., 2011b). On the other hand, in this same fly, male diet affected AGPs capacity to inhibit female receptivity (Abraham et al., 2012). Similarly, in *C. capitata* the ability of sterile males to inhibit female receptivity is greatly improved when they are fed a diet rich with protein (Gavriel et al., 2009; but see Shelly & Kennelly, 2002). In *B. tryoni* feeding males with only 24 or 48 h of yeast hydrolysate after emergence increases sperm numbers and decreases female remating (Pérez-Staples et al., 2008b). Overall evidence suggests that in general ingestion of protein at the adult stage in tephritid fruit flies increases their post-copulatory success and should be incorporated into pre-release diets when possible.

#### *Juvenile Hormone treatment*

Juvenile hormone (JH) is a sesquiterpene, which along with other hormones regulates growth and passage through the early development stages of the life cycle. JH in some species also regulates the beginning of the process of sexual maturation in females, males or both sexes (Ringo, 2002). It has been postulated that this hormone is primarily responsible for coordinating reproductive maturity in *A. suspensa* males and induces early development (Teal et al., 2000). The use of methoprene (an analog of JH) in males of *A. fraterculus* allows reaching sexual maturity at an early stage (Segura et al., 2011). Since the genera *Anastrepha* and *Bactrocera* require several days to reach sexual maturity, the use of JH allows SIT programs to reduce the storage time of the flies in the facility and reduce time in the field to achieve male sexual maturity (Teal et al., 2000). The use of methoprene affects the process of sexual maturation, but in *A. fraterculus* females injected with AGPs of 6 d-old methoprene-treated males had higher receptivity, compared to females injected with AGPs of sexual mature untreated-males (Abraham et al., 2012). This suggests, that methoprene matures males up to a certain point. Methoprene treated young males can mate, but perhaps methoprene did not mature their accessory glands. In this same species, wild females mated with methoprene-treated males had higher remating rate and shorter refractory periods than wild females mated with sterile untreated-males or wild males (Abraham et al., 2013). On the contrary, methoprene treatment alone or accompanied with protein had no effect on female remating propensity in *B. cucurbitae* (Haq et al., 2014). Clearly, this is a topic that deserves further research in a variety of species.

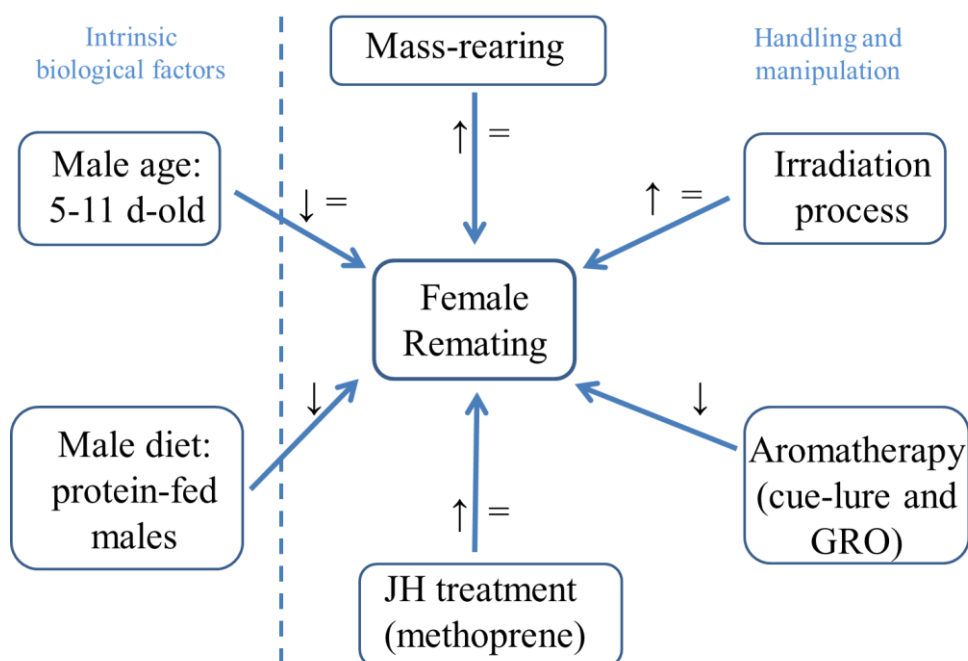
#### *Aromatherapy*

In order to counteract the detrimental effect of mass-rearing process and irradiation, some effort has been made using different substances to increase male mating success. In *C. capitata*, the use of ginger root oil (GRO) increase male mating success (Shelly et al., 2002) and females mated with such males had lower remating rate (Shelly et al., 2004; Morelli et al., 2010). Similarly, females of *B. tryoni* mated with lure-fed males (males fed with plant derived chemicals to enhance their mating competitiveness) had lower remating propensity (Kumaran et al., 2013). Our understanding of how female remating behaviour is modulated by chemicals beyond a few substances tested for aromatherapy is still incipient. Certainly the changes in

female olfactory behaviour observed as a result of the transfer of AGPs during mating, suggests that the relationship between female remating, male pheromones and lures is a complex one. Further research is needed on the interaction between chemical ecology and the sexual behaviour of tephritid flies of economic importance.

### Male age

Male age is an important factor that can influence their pre and post-copulatory success. Sperm storage by females and sperm number decline with male age in *C. capitata* and *B. tryoni*, respectively (Taylor et al., 2001; Pérez-Staples et al., 2008b; but see Papanastasiou et al., 2011 and Costa et al., 2012 for *C. capitata*). In *C. capitata* middle-aged males (11 d-old) are more effective in reducing female sexual receptivity than younger (4 d-old) or older (18 d-old) males (Gavriel et al., 2009). Similarly, Shelly et al. (2007) found that middle aged males (5 or 10 d-old) are more effective, compared to younger males (3-4 d-old). A recent study found that in *C. capitata* female remating was not influenced by male age (from 4 to 20 d-old) when males were well-nourished (Costa et al., 2012). In contrast, in *B. tryoni*, male age does not affect their ability to inhibit female remating (Pérez-Staples et al., 2008b).



**Fig. 1.** Principal factors affecting female remating behavior in tephritid flies of economic importance. Symbols indicate that the factor increases (↑), decreases (↓) or has no effect (=) on female remating, depending on the studied species. JH: juvenile hormone. GRO: ginger root oil.

### Impact of remating in SIT

If sterile males transfer lower quality and/or quantity of sperm and/or AGPs, wild females could remate more often, or earlier, when mating with a sterile male. In *C. capitata* sterile males are less able to inhibit female receptivity (Kraaijeveld & Chapman, 2004; Gavriel et al., 2009; Morelli et al., 2013). Also, 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). A high female remating rate and a rapid renewal of receptivity may compromise the efficiency of SIT, as a female could remate with a wild fertile male, thereby leaving viable offspring (Bloem et al., 1993; Kraaijeveld & Chapman, 2004). Additionally, we know little about how female choice changes after mating with a sterile male, perhaps females could change their choice after mating with a sterile male and prefer a wild male as second partner.

### Future perspectives

Some points could be highlighted as “black holes” in the study of the role of sperm and male accessory glands in the Tephritidae family, due to the difficulty of separating one factor from the other. The use of molecular techniques such as producing AGP-deficient males, RNA interference (e.g. Gabrieli et al., 2016), or the use of genetically modified insects could greatly aid in this endeavour. Also, attempting artificial insemination with only sperm could help disentangling these two factors. A deeper understanding on the physiological and chemical mechanisms that govern female remating as well as how post-teneral treatments affect these physiological processes is needed. For example, a further knowledge gap is if and how both internal and external copulatory courtship influences female remating behaviour.

Furthermore, there are relatively few species where the genes expressed in the male reproductive system and the proteins of the seminal fluid have been characterized in detail. Information acquired through the genome, proteome or transcriptome will also aid our understanding on the function of AGPs (e.g., Scolari et al., 2012; Wei et al., 2015b; 2016). The genomic and transcriptomic data for tephritids remains limited. Despite the evolutionary constraints at the functional level, genes with reproductive functions are evolving faster than other genes not associated with reproduction (Wagstaff & Begun, 2005). Thus, efforts must continue in the search for new genes and proteins in the male and female reproductive tract. In particular, control methods may benefit from finding genes that are differentially regulated in the female reproductive tract in response to mating, and corroborating the transfer of AGPs (transcripts and proteins) from males to females during copulation (e.g., Scolari et al., 2012; 2014; Kumaran et al., 2014). Identification of molecular and genetic mechanisms that are involved in mating inhibition, as well as displacement or incapacitation of ejaculates are also interesting areas to pursue. It will also be useful to compare sequences in other species to find putative orthologs, and to study the post-copulatory molecular interactions between sexes and the molecular mechanisms underlying tephritid reproductive biology.

Our knowledge on female remating is constrained to a small number of species, generally of economic importance. A large number of species have been relatively ignored inside the Tephritidae family and in related families. Many of these species will represent valuable model organisms for the study of cryptic post-copulatory process, such cryptic female choice and sperm competition. One curious example is that of the Agave fly *Euxesta bilimequi* (Diptera: Ulidiidae), where females remate repeatedly, expel the sperm after copulation and

consume the sperm (Rodríguez et al., 2013). This and others insects provide us with the opportunity to study polyandry, sexual conflict, and cost and benefits of these behaviours to females.

## Acknowledgements

We thank Dinesh Rao for comments to the manuscript. Funding was provided by a CONACyT Ciencia Básica grant (no. 179741) awarded to DPS and postdoctoral fellowships from CONICET awarded to SA and from CONACyT to MHC.

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# **Sterile Insect Technique**

## Molecular tools in the evaluation of SIT programmes success against *Ceratitis capitata* in Spain: a review

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### Abstract

**Background:** The success of sterile insect technique (SIT) programs against many tephritid fruit flies, including *Ceratitis capitata* (Wiedemann), relies on the mating success of released sterile males in the field. Since its development in an area-wide concept, this control program is evaluated by the recapture ratio of sterile versus wild flies. This measure neither takes into account the real target of the SIT that is the wild female, nor does it give any clue about the success of released sterile males. Thus, the contribution of released sterile males to reduce the wild population still remains as a key issue. In this work we review recent findings on how sterile males contribute to reduce wild populations by means of analyzing the sperm content of wild females, as the real target of the SIT program.

**Methods:** A mating competition test was initially performed in laboratory and then under semi-natural conditions, with different *Ceratitis capitata* Vienna-8 *tsl* release ratios. The efficacy of the SIT and its contribution to reduce wild population was assessed by determining the percentage of females mated with sterile male, with a sperm ID molecular test, and by linking to offspring production on sentinel hosts.

**Results:** Statistical methods have been developed with the obtained data of sperm ID in the spermathecae of captured females and with data of viable offspring produced in sentinel fruits, revealing that both can be predicted using release ratio and mean temperature. Moreover, humidity arose also as a factor influencing the female capture in lured traps. A strong negative relationship was established between the proportion of Vienna-8 mated females and *Ceratitis capitata* offspring production, being a key point for a model to predict the SIT program success.

**Conclusions:** The statistical models developed should contribute to enhance the efficacy of SIT programs against *Ceratitis capitata* by means of modulation of release ratios by season temperature and by checking wild female's sterile sperm content.

**Keywords:** *Ceratitis capitata*, offspring reduction, statistical models, SIT, sperm ID.

### ***Ceratitis capitata* control in Spain: a historical perspective and current trends**

The Mediterranean fruit fly or medfly, *Ceratitis capitata* (Wiedemann), is a key pest on citrus and other deciduous fruits produced in Spain (Beitia et al., 2003). *Ceratitis capitata* is believed to be of sub-Sahara African origin, which invaded the Mediterranean basin in a first step in 1842 finding the citrus as hosts, and receiving its common name from this first invaded area (Malacrida et al., 2007). This species has a great ability to disperse, to use alternative hosts and has a great developmental plasticity, which allows its survival in tropical and temperate regions (Vera et al., 2002; FAO/IAEA, 2013; Navarro-Llopis et al., 2014). The lack of natural enemies in the Mediterranean region altogether with the behavioural plasticity to avoid them that this species exhibits, may be the responsible for the outbreaks that give to this species its key pest status (Liquido et al., 1991; Beitia et al., 2003; Malacrida et al., 2007; Argov & Gazit, 2008; Sabater-Muñoz et al., 2009).

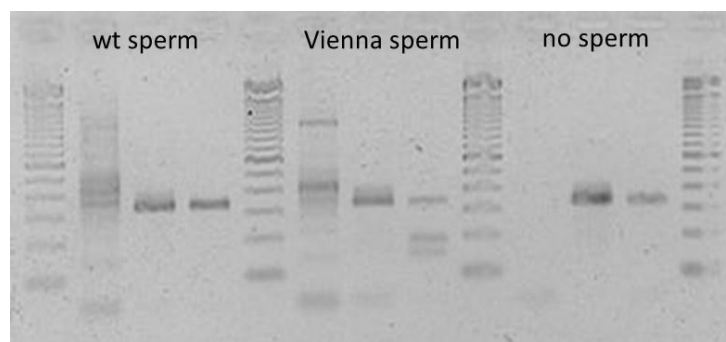
In Spain, and more precisely in the Valencia region, during the past decades the control of this fruit fly species has relied primarily on chemical control. However, the serious threat to the international trade market caused in 2001, linked to the appearance of malathion resistant populations (Magaña et al., 2009) and the establishment of the European normative 2009/128/CE and Horizon 2014-2020, led to the implementation of environmental safe techniques within the Integrated Pest Management (IPM) program established to control this pest (Beitia et al., 2003; Sabater-Muñoz et al., 2009; Urbaneja et al., 2010-15). In 2003 an area-wide IPM program was set-up by the Valencian Community government involving environmental safe procedures such as mass trapping, chemosterilization traps, surveillance nets, and the creation of a mass-releasing facility for the implementation of the Sterile Insect Technique (SIT) against this species. The SIT is based on the birth control system exerted by released sterile males. This birth control has been successful in several countries against multiple species (Dyck et al., 2005 and references herein). The characterization of parasitoids and potential predators was also a key point in this area-wide IPM program that promoted the use of these disvalued cultural practices, during the late century (Beitia et al., 2003; Monzó et al., 2007a,b, 2010, 2011; Urbaneja et al., 2010-15). In 2006, two facilities were set-up in the region, one for mass-rearing and another for mass-releasing which cover the nearly 182,000 Ha of citrus production. This citrus production area harbors more than 35 citrus varieties that are mainly for fresh fruit export market; a market that requires zero residues in fruit and no pest presence. The actual core of this IPM in the Valencian Community is the application of the SIT throughout the citrus production area. The program is funded by the community government whereas the mass-trapping and chemical control rely on the farmers with the aid of the GIP citricos web tool (Urbaneja et al., 2010-15).

### **The Sterile Insect Technique: principles, application and evaluation**

The SIT relies mainly on the birth control exerted by released sterile males on the target population (Dyck et al., 2005). The released sterile males should mate with the wild females

and block the reproduction of the species, as the sterile sperm carry lethal dominant mutations that stop the development of viable next generation, imposing a birth control on the population and henceforth reducing its numbers. Its application requires of three steps (FAO/IAEA, 2003; Dyck *et al.*, 2005): 1) ability to rear, sterilize and release enough insects to overflow the area with a high sterile:wild ratio; 2) the released sterile males should compete and mate with their wild counterparts; and 3) a monitoring system to follow up points 1 and 2, and a system to identify the sterile males. Nowadays the SIT is applied under the area-wide SIT umbrella, as the best option to control a certain pest species in a determined production area. Many species are the target of SIT (mosquitoes, lepidopterans, flies, screwworms, etc) but tephritid flies species remain as the key species both as good examples of success and as the best target species for SIT.

Despite the great success of SIT in the control of tephritid flies, the evaluation of the success of SIT against medfly relies only in the recapture ratio data. By using tridmelure baited traps the ratio sterile: wild of males can be determined after identification of sterile insects (Weldon, 2005), and when this ratio is 100:1 or higher, it is said that the SIT program has succeeded. But this ratio does not take into account the real target of SIT that is the wild females (Hendrich *et al.*, 2002). In the past McInnis and coworkers (1993, 1994) also noticed this problem, solving it by making measurement of sperm heads accumulated in the female spermathecae or by determining the induced sterility index in the population by measuring the number of viable and unviable eggs in egg clutches in fruits in comparison with untreated areas. Even though both methods are a direct measure, they are long and tedious to put in practice on a daily manner. In 2007, our group (San Andrés *et al.*, 2007) established a new methodology to determine the presence of sterile sperm in the female spermathecae, based on PCR-RFLP patterns (Fig. 1), shedding a new light on how to overcome this problem. Recently, we have improved this methodology (Juan-Blasco *et al.*, 2013a,b,c) to reduce the handling time and improve the sperm ID accuracy.



**Fig. 1.** SpermID method: PCR-RFLP pattern observed for *Ceratitis capitata* females mated with wild type males, sterile (Vienna) males, or unmated. Differences in the pattern allow to identify the sperm in the female spermathecae. For further explanation, see San Andrés *et al.*, 2007.

### **Linking mating success of released sterile males with population reduction and fruit damage avoidance**

The improvement of sterile males' success by boosting their mating achievement (Juan-Blasco et al., 2013a and references herein), and of the release procedures have rendered the aw-SIT a powerful environmentally friendly tool to control the medfly. However, despite the first studies (see Dyck et al., 2005 and references herein) no relationships between released sterile males, population and fruit damage reduction have been clearly established. Here we present a summary of the association observed among sterile males mating success (measured as sterile sperm identified in wild females' spermathecae), and the reduction of the offspring reduction and fruit damage (Juan-Blasco et al., 2013c, 2014). By means of semi-field trials, we have established a relationship between mating achievement, offspring production and fruit damage (Fig. 2). These studies included several fruit species, and different climatic conditions. Mating achievement has been determined by means of application of the SpermID method, as a direct measure of released males' success. The positive identification of sterile sperm in the female spermathecae was achieved even at 1:1 ratio (sterile: wild males) and at all natural conditions tested, opening a great window for the establishment of a direct measurement of SIT program success.



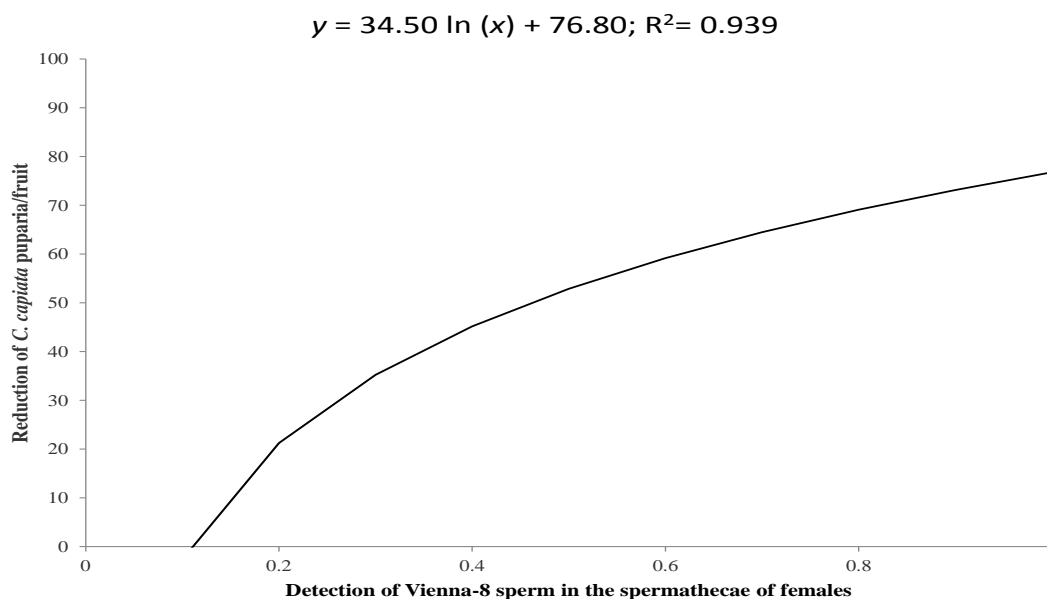
**Fig. 2.** Semi-field trial design. Single caged 20-years old clementine trees were used for testing sterile males' releases and spermID. In each tree, a Tephri-trap was hold, with 10 sentinel fruits (apples). Data from number of affected apples, and puparia obtained from each apple was recorded. Each captured female was subjected to spermID test by PCR and the presence of sterile sperm was recorded.

The proposed model has established for the first time a correlation between the percentage of sterile male-mated females and population reduction (Fig. 3). This model has enhanced the use of molecular techniques such as the PCR-RFLP in biological control of pest insects. Moreover, it has become a valuable tool for SIT programs mainly by indicating the success of



released males in achieving their role, to mate with wild females and by reducing the next generation.

The application of this sperm ID method to samples captured in the aw-SIT program has unveiled for the first time, that in some parts of the Valencian Community 50% of captured females were mated by the released males, indicating a correlated 50% of population reduction. Yet, these results require further analysis.



**Fig. 3.** Integrated correlation method describing the relationship between percentage of females mated by sterile males (indicated as percentage of females with sterile male sperm in their spermathecae) (x) and offspring reduction and fruit damage reduction (y).

## Final Remarks

The SIT is one of the best environmental friendly tools for *Ceratitis capitata* control. Cost-benefit analyses have demonstrated the convenience of applying this control technique in an area-wide concept. The sperm ID method presented here is a valuable and direct measure of sterile males' success in open field conditions that contributes to a reduction of operational costs and therefore its adoption in different aw-IPM programs that relies in the SIT is highly recommended.

## Acknowledgements

The authors would like to acknowledge two colleagues at Smurfit institute of Genetics (Trinity College of Dublin) for manuscript improvement. This work has been developed within the framework of the research project of the Spanish Ministry of Economy and

Competitiveness project no. AGL2010-21349-C02-02, the Valencian Community research project GVPRE/2008/323 and IAEA-FAO Research Contract 17516.

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## **New Mediterranean fruit fly emergence and release facility at Tapachula, Chiapas, Mexico**

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### **Abstract**

*Background:* Sterile Insect Technique (SIT) programmes have two main areas of activity. First, the mass-rearing of insects which is a specialized activity and any variation has a significant impact on the quality of reared insects. Second, the post-production activities, involving packing, emergence, holding and release of sterile flies, is also a specialized procedure and requires similar but different needs, like demands for space and movement of adult flies.

*Methods:* A multidisciplinary group of experts on SIT, industry and construction was formed to design a new fly emergence and release facility, having in mind, the needs of the programme and the best available technology. The construction of fruit flies fly emergence and release facility, should include, among others: a) selection of the site; and b) design of the building, logical working flowchart, packing system, capacity and equipment.

*Results:* The new Mediterranean fruit fly emergence and release facility has a capacity to manage 1200 million flies, per week. It has been designed with state of the art technology to control environmental conditions like, temperature and humidity to guarantee an optimal development of the flies in each one of the areas: reception of pupae, 12 fly emergence rooms, 4 chill rooms, diet preparation and quality control rooms. Maintenance, warehouse and water treatment areas were taken into consideration, as well as an energy saver system. The packing system is conducted with the specially designed Mexico tower type, which assures a better quality and management of the biological material, the amount of pupae per tray is distributed by specific equipment for an accurate weight and 2 conveyor bands. The Mexico tower type is conformed to 16 trays, having each one, 2 devices with 40 g of food, 2 plastic resting areas, a pillow to supply water and a pupae container to hold 65,000 pupae. Air conditioning system includes integrated equipment of 25 tons of refrigeration, dehumidifier with a capacity of 60 l/day, especially designed to control the 21-23 °C needed in each emergence room. Cold rooms are equipped with three 48,000 BTU air conditioning compressors and six 24,000 BTU evaporators, as well as one 1400 m<sup>3</sup>/h air dehumidifier. Materials used in this fly emergence and release facility are reusable and because of that, an especially designed industrial washing machine is used to clean them.

*Conclusions:* The conditions under which the fly packing activities are conducted are as relevant to the overall success of the SIT activities as those of the production of high quality sterile insects. Attention at the fly emergence and release facility should include the needs of

the flies in each one of the different areas as well as the impact of the activities to the environment.

*Keywords:* *Ceratitis capitata*, environmental conditions, fly emergence, fly handling

## Background

The Sterile Insect Technique (SIT) is a birth control method that relies in the release of an overflooding ratio of sterilized males to compete with the wild ones for mating with wild females (Dyck et al., 2005). To achieve this population control, as explained, males of the target species should be produced, sterilized and released in a massive format. The design of a packing, holding, chilling and release facility is of primary importance in the application of the SIT. Since its development in late 50's, the technique has required improvements in all the stages, from design of mass-rearing facilities, to sexing strain development and to the design of mass-release devices (Dyck et al., 2005, and references herein). Nowadays, for the most important Tephritidae pest species, mass-rearing facilities are well designed, requiring only its adaptation to final production capacity and to locally available resources. Indeed, Tephritidae control programs in America, Europe, and Africa have initiated their activities in adapted warehouses or existing buildings according to the size of the amount of flies to be released, creating different environments according to the packing, holding and release methodology needed for each fruit fly species.

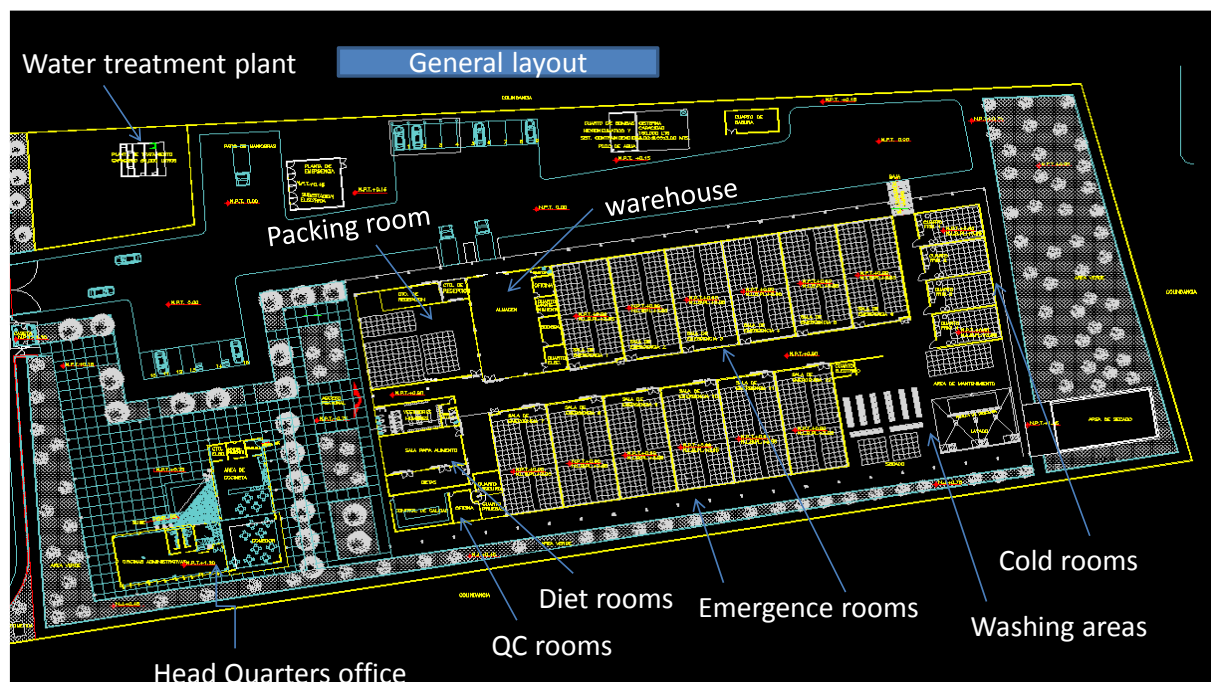
The experience in Mexico, for this purpose, involves the use of four different types of constructions. The first was an existing building, at about 2 kilometers from the airport, used to pack the flies in aluminum cages and hold them in normal rooms with window air conditioning packages and then taken to a chilling room to prepare the flies for aerial release. A few years later, it was changed to the release-bag system and because of the space needed, the packaging place was moved to an existing bigger warehouse. Years later a new warehouse was built close to the Mass Rearing Facility, with new equipment to accommodate the bags and facilitate the transport to the airport at 40 kilometers of distance. It was then decided to move again to another existing warehouse, close to the airport (at about 15 kilometers) (Zavala, 2008; Gutiérrez et al., 2010.) Years before the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) regional program constructed a new packing facility in Guatemala, under the guidance of the Department of Agriculture of the United States (USDA). Finally, a decision was taken to build a new packing center, specially designed for that purpose, back in Mexico.

The objective of this work, was to show the design of the new mass-rearing and mass-releasing facilities in Mexico.

## Design and description of a Medfly packing center

The construction of Tephritidae fruit fly Packing Center should include among others five key points:

- Selection of the site: (i) should be placed strategically within the working area, to avoid excessive management and time of ferry for the distribution of the flies; (ii) close to an airport, because the aerial release, under the adult chilling system includes the accumulation of flies in a refrigerated box and can cause compaction during the ride to the airport; (iii) basic services available, it is important to have public electricity, water supply and within an industrial area in order to have spare parts for the machinery in use; and (iv) accessibility, with roads.
- Packing system: at the present time there are 4 basic packing systems bags, PARC boxes, sleeves and towers.
- Capacity: depending on the packing system selected, the space and technical requirements will be different.
- Equipment: to cover the needs for each one of the technical areas, such as temperature, humidity, and quality of the air.
- Building design: a logical working flowchart, according to the technical and supporting areas should be performed (Fig. 1).



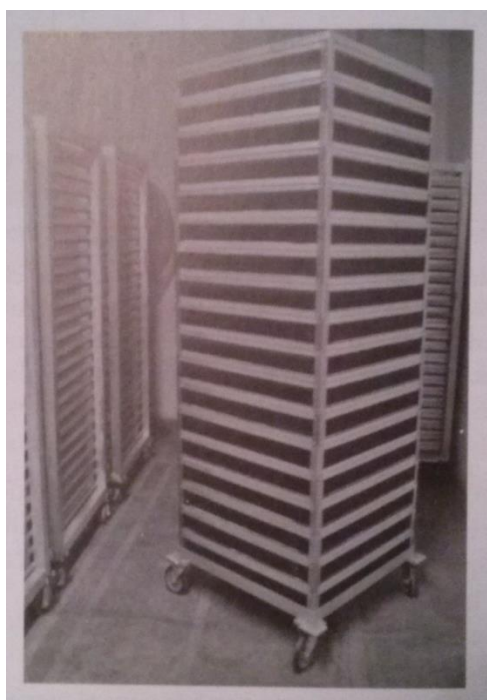
**Fig. 1.** General layout of the Mediterranean fruit fly packing, emergence, chilling and release facility in Tapachula, Chiapas, Mexico.

### Medfly Packing and Emergence systems

Overcrowding is of great concern in mass-rearing facilities. It was normally recommended that medflies were at a density of 2 flies per square centimeter (FAO/IAEA/USDA, 2007). But flies fly and also needs resting places. So, to obtain an excellent batch of sterile medfly males (high flight ability, mating performance, etc) it was needed to define the amount of flies per packing unit, while keeping a high resting place; or at the inverse, depending on the resting place available, the amount of pupae should be calculated.

In this new facility we used the tower system for emergence, and is called Mexican Tower (MT) (Zavala et al., 2010). Each Mexican Tower has 16 levels of 82 cm large, 70 cm wide and 10 cm high (Fig. 2), which gives a level surface of 14520 cm<sup>2</sup>. Each level contains 2 food dispensers, 1 pillow for water supply, 1 pupae container and 2 plastic resting areas (of 120 cm large per 40 cm wide; which gives a resting surface of 19200 cm<sup>2</sup>) for the emerged adults. At each pupae container are deposited 55000 pupae, which considering a 90% emergence, we obtain a rearing rate of 1.47 medflies per square centimeter.

Medfly allowed to emerge in these MT, are subjected to QC following IAEA procedures (FAO/IAEA/USDA 2013).



**Fig. 2.** Mexican emergence type tower (MT). This system composed of 16 levels allows an easy transfer of devices between rooms, while keeps medfly flies with comfortable space (1.47 medflies per cm<sup>2</sup>). Picture from Zavala et al., 2010.

### Emergence room

Once determined the emergence cage design, the next step is the design of the emergence room. A medfly mass rearing facility should have enough space to hold all production till packing day, which usually is at 3-days-old age. Our mass rearing facility produces 1000



million medflies per week, meaning 142 million flies per day. This production volume requires 161 Mexico type towers per day (as indicated previously, 55000 pupae per level, and 16 levels per tower, means 880000 pupae per tower). As each MT tower occupies 1 square meter, an emergence room holding one complete production day, requires at least 161 m<sup>2</sup>.

The design should also take in consideration that flies should be kept at dark (a quiet place), with concrete temperature, humidity and air quality conditions. But, also depends in an additional criteria, the quietude of the room. When holding the emergence towers in a big room which is opened daily to introduce new towers or to remove the oldest ones, flies are subjected to stress. To avoid this issue, the Mexican facility has decided to have one emergence room per production day (of about 161 m<sup>2</sup>), also because it is easier to manage temperature, humidity and air quality in small spaces than in large areas to assure the quality of the flies.

Temperature control in the emergence or holding rooms is of primary importance, especially in tropical areas (like Mexico), but even in cold environments, humidity and air exchange also require management to preserve the quality of the flies. At the new facility temperature, humidity and air quality are managed by 2 integrated air conditioning packages of 25 ton (440V, 3F-4H, 60HZ, 10,000CFM) and 1 drying dehumidifier of 120 pints/day (127V, 1F-2H, 60HZ, and 250 CFM), which altogether allow more than 10 air interchanges per day to assure air quality.

### **Chilling rooms**

As in the emergence or packing rooms, the control of the environment in chilled rooms plays an important role. The equipment in each chilled room is composed by: three 48,000 BTU Condensers (220V, 3F-4H, 60Hz), six low profile 24,000 BTU evaporators (220V, 2F-3H, 60Hz, TSS -4°C 2,800 CFM), one 1,400 m<sup>3</sup>/h air processing dehumidifier and 1 drier (440V, 3F-4H, 60 HZ). This equipment will allow maintaining a stable temperature in each tower (temperature for flies immobilization for handling and transferring to release devices) and for maintaining flies humidity (excessive fly humidity can cause flies stacking at release device dispensator, blocking the release), while keeping the higher quality parameters for released sterilized males.

The amount of flies per release day and per release device will determine the number of chilled rooms required per day. The new facility has 4 chilled rooms, each for a maximum capacity of 25 million flies per load. Whereas each release airplane needs 3 release devices, each one of 20 million flies' capacity.

Each fly batch is also tested (flight ability, number of walking flies, ...) after packaging in release devices, by using the airplane release system, at the airport.

### **Reception, packing and Quality control rooms**

A set of Routine and periodic quality control tests are required to determine the effect of packing, holding, chilling and release (reviewed at Zavala et al., 2010; FAO/IAEA/USDA

2007, 2013) as well as to verify that the sterile insect received fulfill minimal requirements as specified in the Manual for Product Quality Control for Sterile Mass-Reared and Released Tephritid Fruit Flies (FAO/IAEA/USDA, 2007, 2013). Quality control tests (pupae weight, emergence percentage, flight ability, mating performance, ...) are conducted during different steps of the process at the emergence and release facility and these rooms should comply with the same environment conditions as the emergence rooms.

In addition, at the packing room there is a special equipment for the evenly distribution of the pupae in the containers placed at each level of the MT tower. This equipment, a linear vibrator (of power 1KW, 220 V, 2F-3H, 60 HZ) with 10 distribution heads, allows a programmable operation of volumetric distribution at 70 doses (from 10 to 3000 grams of pupae) per minute with an accuracy of 1.5 grams.

### **Supporting areas**

We classified as support areas all the remaining rooms as warehouse (one for supplies, other for equipment), diet preparation room, washing, and all other areas on which flies are not kept, but are essential for the correct operation of the facility (including offices and restrooms). One area of special requirements is the washing room, as mechanization of this area reduces in 40% the potable water requirement and helps in the reduction of sewage waters (with its economic impact).

### **Water treatment equipment**

Packing facilities usually generate sewage waters from two points: sanitary (from offices and restrooms) and industrial processes (from diet preparation, to washing of all cages, towers, etc). As the potable water consumption in all the facility is great, a special water treatment equipment was planned. This involves an extended aeration of soluble organic material containing waters, with the use of bacterial treatment to reduce the quantity of activated humus. The obtained treated water is re-used at the washing rooms or for gardening in all around the facility, while complying with National environment legislation.

### **Conclusions**

The conditions under which the fly packing activities are conducted are as relevant to the overall success of the SIT activities as those of the production of high quality sterile insects. Attention at the fly emergence and release facility should include the needs of the flies in each one of the different areas as well as the impact of the activities to the environment.

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## **Flight ability and survival during the holding, chilling and aerial release of two *Anastrepha ludens* (Diptera: Tephritidae) sterile fly strains**

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### **Abstract**

*Background:* Flight ability and survival are crucial quality parameters for mass-reared sterile insects. Emerging and Release Facilities (ERFs) for sterile insects require that both parameters be estimated uniformly at the different stages of the process, which include the holding, pre-chilling, post-chilling and post-release of the sterile insects.

*Methods:* We evaluated a device made of a PVC hydraulic connection of 10 by 8 cm (diameter by height) which was closed at the bottom with a 9 cm in diameter Petri dish. In the top, we used a metal mesh to allow ventilation for fruit fly adults which was removed after the placement of a black PVC pipe 9 x 10 cm (diameter by height) to test flight ability. This device was placed inside the packing units (screen tower type-Mexico) for adult emergence, and was retired after each of the four above mentioned stages. The control treatments consisted of the routinely methods applied in the ERFs to estimate flight ability and survival.

*Results:* Values of flight ability were significantly lower using the PVC devices than with the current methods. This was attributed to a possible lack of ventilation provoking some accumulation of humidity and other waste products; conversely, survival values were more consistent and uniform using the PVC device during the tests. Flight ability also showed significant differences among the different stages of the process, demonstrating that fruit fly adults suffer cumulative and deleterious effects from handling procedures.

*Conclusions:* The device tested is a prototype that uses simple, practical and inexpensive materials that allow the traceability of both parameters at each stage of packing and upon the release of the sterile flies. With some modifications to improve the air ventilation, this device could be used to routinely monitor the quality of sterile insects released into the field.

*Keywords:* bisexual and genetic sexing strains, chilling process, sterile flies, SIT, quality of sterile flies.

### **Introduction**

Fruit fly control and eradication programs require advanced technologies in order to achieve their objectives. One of these technologies entails large-scale release of sterile male flies aimed at mating with wild females in the field and induces sterility in the population (Cunningham et al., 1980; Klassen et al., 1994; Lance et al., 2000). To reach this goal it is essential to have a mass-production system of sterile insects which ensures their quality. A second requirement is an efficient emerging and a field-releasing system for mass-reared

sterile male flies. In order to guarantee that sterile insects can successfully compete against wild males, it is important to carry out a series of routine evaluations (i.e., quality control tests) to verify if sterile males are fulfilling their objectives.

Some of the most important parameters to evaluate sterile males' quality and their performance in the field are flight ability and survival. Flight ability is defined as the adults' capacity to fly (FAO/IAEA/USDA, 2014; Calkins & Parker, 2005). This parameter is of great significance because it shows the quality of the insects after mass-rearing, irradiation, packing, emergence, sexual maturation and release processes (Villaseñor et al., 2010). Survival in the field is, at least in part, a result of food quality during the insect sexual maturation before release, and it hints the possible persistence in the field under conditions of water and food shortage (FAO/IAEA/USDA, 2014; Calkins & Parker, 2005).

The traditional methodology to determine each of these parameters is carried out at the Emerging and Release Facilities (ERF) after pupae are received and after insects have gone through the chilling process, before release. However, it is necessary to evaluate these parameters in each stage of the holding and release process (see 2007 FAO manual). According to Zavala et al. (2010), the stages are the following: 1. when pupae are received in the ERF, 2. on sexually mature adults before chilling, 3. Post-chilling, and 4. Post-release of sterile adults in the field. In this study we evaluated a new device made of PVC pipes of 11-cm-dia (see Fig.1) based on the methodology developed by Montoya et al. (2013) to determine the flight ability and survival of mass-reared sterile insects during the process of holding, chilling and field release in SIT programs.

## Materials and Methods

### *Study site*

Evaluations were carried out at the Mediterranean sterile flies ERF (CEMM, by its acronym in Spanish) (SAGARPA-SENASICA), and at the Methods Development Unit of the Moscafrut program-SAGARPA-IICA located in Metapa de Domínguez, Chiapas, Mexico.

### *Insects*

*Anastrepha ludens* flies from one bisexual strain and one genetic sexing strain (GSS) Tapachula-7 were tested. Insects were received as irradiated pupae and handled according to the Release and Packing Procedure Manual (SAGARPA, 2012).

### *Device*

A PVC device was used (based on a Petri dish-type device that was designed and tested for *Ceratitis capitata* by Montoya et al., 2013) which consisted of a modified PVC hydraulic connection (10 cm in diameter), with some *ad hoc* adjustments (Fig. 1). This device was closed at the bottom with a Petri dish-type of 9 cm, and a removable top made with fly insect mesh to allow ventilation for fruit fly adults and subsequent placement of a black PVC pipe 9 x 10 cm (diameter by height) to test flight ability (FAO/IAEA/USDA, 2014).



**Fig. 1.** PVC device used to evaluate flight ability and survival of sterile insects. Tests were performed at the Emergence and Release Facility in Tapachula, Mexico: A) PVC hydraulic connection 11 cm of diameter to hold emerged fruit fly adults. B) PVC black tube of 9 cm L to test flight ability. C) PVC tube with compartments for the pupa, food and water. D) Fly insect mesh to prevent fly adults escape during holding. E) Polar view of the PVC device with the insect mesh. F) Black tube connected to the PVC device to perform the ability test after remove the insect mesh.

### Evaluations

We prepared three levels of the Mexico-type fly holding tower (see Hernandez et al., 2010) per replication, where 20,000 viable pupae from one of the two tested strains were placed per level (SAGARPA, 2012). On each level, a pad saturated with  $\approx 230$ -ml of water, and two wire ways each with 60 g of food prepared with a mix of hydrolyzed protein (yeast hydrolyzed enzymatic, ICN Biochemicals, Aurora, OH) and sugar (1:24) were placed (Liedo et al., 2013). Five days after 50% emergence of adult flies, they were subjected to the chilling process at  $2 \pm 1$  °C for 45 minutes and subsequently were air released into the field. Flight ability and survival parameters were determined through the four stages of the process as follows:

#### Flight ability

Four PVC devices were prepared per replicate corresponding to each stage of the process (i.e., pre-holding, pre-chilling, post-chilling and post-release). In each device, 100 pupae were placed. The devices carried on food and water in specially designed compartments (Fig. 1C), with exception in the pre-holding stage where flies were free to abandon the devices as they were emerging. Flight ability was calculated as the percentage of flying flies collected at each stage of the process:

*a) Pre-holding.* No water and food were provided in this evaluation. The top section of the device (Fig. 1A) was kept free and connected onto the black tube (Fig. 1B), which was covered with 5 cm from the top with talc. The devices were placed under fluorescent lighting of about 1500 lux of light intensity. Upon emergence, flying flies stimulated by the light escaped the tube. The number of non-flying and deformed flies, as well the number of empty pupae (emerged flies), were recorded. The control treatment consisted of the routine

estimation of flight ability performed at the Quality control laboratory as described in manual FAO/USDA/IAEA (2014). The percentage of flying flies from the device and the control method were estimated as follows: Flying flies = (% emergence) – (% of deformed + non-flying) flies.

*b) Pre-chilling.* In this stage, the device was sealed with the mesh and placed in the interior of one of the levels of the Mexico-type tower. Five days after 50% of the flies had emerged, the device was removed from the level, the mesh was removed and the black tube with talcum installed. The complete device was placed in light conditions (as previously described) to allow capable flies to fly from the tube. The percentage of flying flies was calculated. In the control treatment, flight ability was determined taking into account the amount of flies collected from one level. After flies reached sexual maturity and before pre-chilling process, the level was placed under lighting for approximately 10 minutes to allow flies to fly; and the remaining flies were weighed. The value of flying flies was calculated as the difference in the initial weight of the pupae placed on the level and the residual pupae collected (non flying flies). From the collected pupae we obtained the percentage of absolute fliers (see details on Sterile Fruit Flies Quality Control Manual, SAGARPA, 2012).

*c) Post-chilling.* Two devices were placed in one level of the tower. One of them was used to test this stage and the other one was used for the post-release stage. Both devices together with the packed flies from that level were chilled at  $2 \pm 1^{\circ}\text{C}$  for 45 minutes. After chilling, one of those devices was recovered, the mesh removed and the black tube installed. Emerged fly adults were stimulated by lighting to escape from the tube. Flight ability was estimated as previously described. Control evaluation involved the determination of absolute fliers through the level following the same method previously cited.

*d) Post-release.* After chilling, the last device was placed into a release-cage hold by hangers to prevent it from falling apart during the aerial transport and release process. As the control treatment, we took a sample of 100 chilled flies and put them in a 20 x 15 cm bag made of fabric tulle, sealed with Velcro and placed close the PCV device. Both samples were recovered from the plane after release. The device was placed in the interior of a 30x30x30 cm clear acrylic cage under laboratory conditions and below fluorescent lighting to stimulate flies to fly. The bag containing 100 flies was unlaced over a 35 x 27 cm plastic tray so all flying flies could escape the tube. Nonetheless, remaining flies were counted as non-flying flies, deformed ones and dead ones. Flying flies percentage was calculated with these data.

### *Survival*

*a) Pre-holding.* All tests were done under laboratory conditions. Initially, 100 pupae were placed in the PVC tube. When the adults emerged, the flies that were able to fly from the device were collected, counted and placed in a 150 mm-diameter by 25 mm: height plastic petri dish, without water and without food. The number of dead flies was recorded every 12 hours, and the average time (hours) to 50% of survival was determined. The control treatment consisted of a selection of 100 pupae from the same batch as those used in the device, which

were individually placed in a Rejilux 100 cells of  $\frac{3}{4}$  by  $\frac{3}{4}$  by  $\frac{3}{4}$  cm. The number of emerged flies and dead ones was recorded every 12 hours until 50% mortality. We estimated the average time (hours) for 50% surviving adults.

*b) Pre-chilling.* In this stage of the process, the PVC device was placed in a 30x30x30 cm clear acrylic cage and the mesh removed in order to adjust the PVC black tube. All flying flies that could escape from the tubes were placed in a 150 mm-diameter x 25 mm: height Petri dish-type, as previously described. Control treatment consisted in capturing a sample of 100 adults while fluttering and placed them in a 30x30x30 cm clear acrylic cage that was placed on the tower level before chilling. Such sample was allocated in a 150 mm-dia x 25 mm: height Petri dish without water nor food. The number of dead flies was recorded every 12 hours. We estimated the average time (hours) for 50% surviving adults.

*c) Post-chilling.* In this stage, we use the same methodology as in the pre-chilling test but the PVC device passed through the chilling period. For the control treatment, a 20 ml sample of chilled flies was taken from the level tower, placed in a 30x30x30 cm clear acrylic cage and transferred to the laboratory to allow flies to wake up. From this sample, 100 flying flies were taken and placed in a Petri dish of the same dimensions. Flies' mortality was evaluated every 12 hours.

*d) Post-release.* Prior to the chilling process, both the device and the bag made of fabric tulle with the 20 ml of lethargic flies were placed inside the release-box as described before. After release, the device was assembled along with the black tube covered in talcum; to allow capable flies to fly out from the tube. They were placed in a 150-mm-dia x 25-mm: height Petri dish, under laboratory conditions. Mortality was recorded every 12 hours. The tulle bag used for control treatment was opened in a 30x30x30 cm transparent acrylic cage so that 100 fly adults capable of flying can be collected and placed in a Petri dish of the same dimensions. The number of dead adults was recorded every 12 hours. In each stage of the packing process, survival indicator was carried out without water or food (stress).

### *Data analysis*

Two-way ANOVA (evaluation method \* stage of the process) was used. Replication effects were considered to be a random factor within the analysis, and means were compared through a Tukey test ( $\alpha = 0.05$ ) [JMP v. 7 (SAS Institute, Cary, NC, USA)]. Values in percentages were arcsine transformed before the ANOVA analysis.

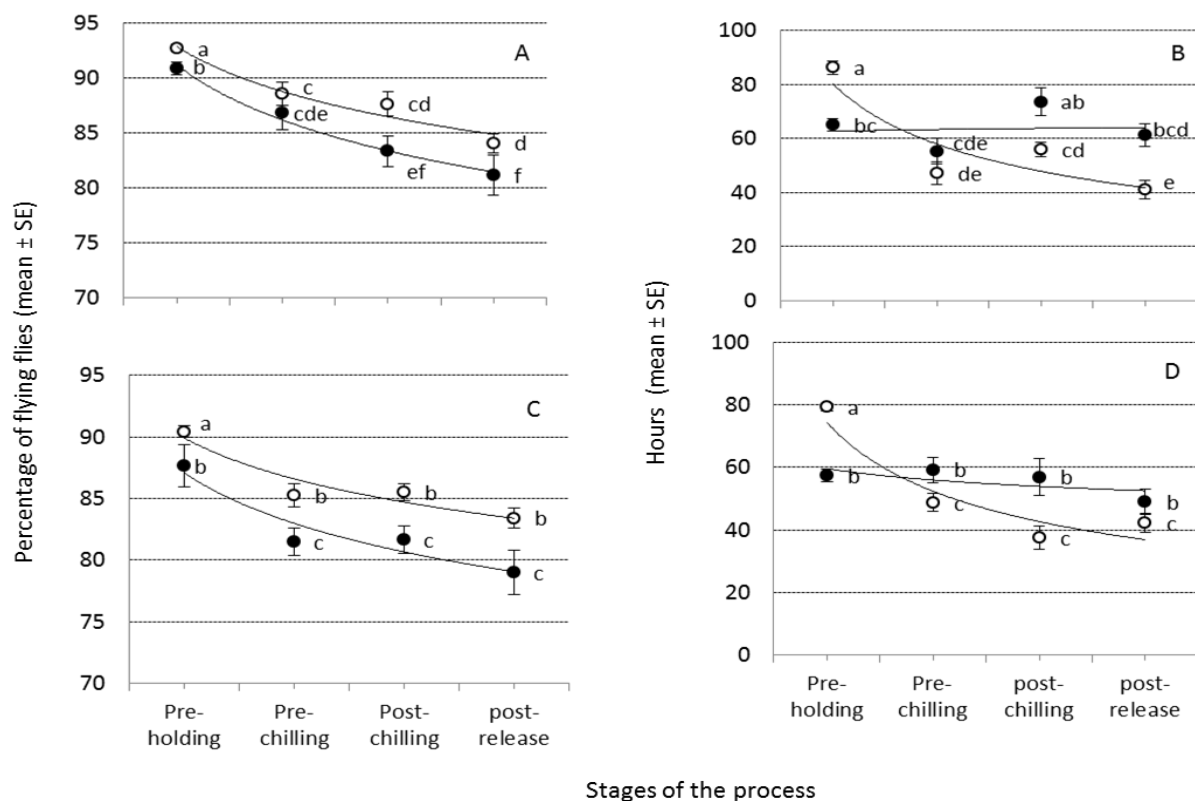


## Results

### Flight ability

**Bisexual strain.** Higher percentages of flying flies were obtained using the control methods than those estimated from the device ( $F_{1, 88} = 10.77$ ;  $P < 0.0015$ ). The stage of the process also had influence on this parameter ( $F_{3, 88} = 20.94$ ;  $P < 0.0001$ ). Higher percentages of flying flies were collected in the holding stage followed by those in the pre and post-chilling stages, but the differences among them were not significant. The post-release stage presented the lowest values but difference with the post-chilling stage was not significant. The interaction between the evaluation method and the stage of the process was not significant ( $F_{3, 88} = 0.561$ ;  $P = 0.6424$ ) (Fig. 2A).

**Tapachula-7 strain.** As in the bisexual strain, higher percentages of flying flies were obtained in the control treatments than those estimated with the PVC device ( $F_{1, 88} = 24.80$ ;  $P < 0.0001$ ). The stage of the process and the release also had influenced this parameter ( $F_{3, 88} = 18.60$ ;  $P < 0.0001$ ). The pre-holding stage showed the highest percentage of flying flies compared with the other stages. The interaction between the evaluation method and the stage of the process was not significant ( $F_{3, 88} = 0.276$ ;  $P = 0.8428$ ) (Fig. 2C).



**Fig. 2.** Percentage of flying flies and survival in hours (mean ± SE) of *A. ludens* bisexual adults (A and B respectively) and *A. ludens* Tap -7 adults (C and D respectively). Both were obtained from the evaluation methods, e.g., control (○) and PVC devices (●) along the packing and release processes. Values marked with the same letter are not significantly different (Tukey  $P \leq 0.05$ ).

### Survival

*Bisexual strain.* Survival from the PVC device was higher than those obtained with the control methods ( $F_{1, 88} = 6.92$ ;  $P < 0.01$ ). The stage of process had a significant influence on this variable ( $F_{1, 88} = 25.07$ ;  $P < 0.0001$ ). In the first stage of the process, higher periods of longevity were registered followed by those in the post-chilling process; but a minor longevity was observed in the post-release stage. The interaction between the evaluation method and the stage of the process was significant ( $F_{3, 88} = 15.99$ ;  $P < 0.0001$ ) (Fig. 2B).

*Tapachula-7 strain.* For this strain, the evaluation method did not affect survival ( $F_{1, 88} = 3.45$ ;  $P = 0.06$ ). Among the different stages of the process, there was a significant effect on this variable ( $F_{3, 88} = 28.11$ ;  $P < 0.0001$ ). The pre-holding stage had the highest values followed by those in the post-chilling and post-release stage. The pre-chilling stage presented the lowest values that did not differ from the post-release stage. The interaction between the evaluation method and the stage of the process was significant ( $F_{3, 88} = 21.04$ ;  $P < 0.0001$ ) (Fig. 2D).

### Discussion

Results in this study indicate some differences in the measured parameters according to the method of evaluation used. In the flight ability test, the percentages of flying flies were greater in the control method. On the contrary, adult survival was higher when using the PVC device.

Studies with *Ceratitis capitata* (Gaskin et al., 2002; Shelly et al., 2013), *A. obliqua* and *A. ludens* bisexual strain and Tapachula-7 strain (Hernández et al., 2010; Arredondo et al., 2015) indicate that confinement density is one of the factors with highest influence on flies' flight ability. As the resting area per individual ( $\text{cm}^2/\text{fly}$ ) increase in the container, the negative effect of the confinement should be lower (Hernández et al., 2010). However, the observed values of flying flies with the PVC device were lower than in the Mexico-type tower, even when in these former devices the packing density was three times lower than in the later ones ( $0.47 \text{ flies}/\text{cm}^2$  vs  $1.3 \text{ flies}/\text{cm}^2$ , respectively). This needs further analysis given we expected that percentage of flying flies obtained with PVC devices were not less than those in the control treatments. Poor ventilation could be the reason for this unexpected result, since the device is a small-closed cylinder that only offers ventilation at the top ( $39 \text{ cm}^2$ ), while in the levels of the Mexico-type tower, ventilation goes throughout the entire surface of  $\approx 14,000 \text{ cm}^2$ . If ventilation is limited, then humidity and carbon dioxide or ammonia can increase (FAO, 2007), affecting flight ability of flies that emerge and are kept in the PVC devices.

The results from both evaluation methods indicate that percentage of flying flies decreases each step, as the releasing process moves forward. It is accepted that in each of the stages of the process, flies come to face factors like confinement density, chilling, and adult compression in the release-box, which adversely affect the values of this parameter (Gaskin et

al., 2002; Salvato et al., 2003; FAO, 2007; Hernández et al., 2010; Reynold & Orchard, 2011; Andress et al., 2012; Shelly et al., 2013).

Survival is a parameter highly related to the diet provided to adult flies (Blay & Yuval, 1997; Yuval et al., 2007; Pérez-Staples et al., 2007; Reynolds & Orchard, 2011; Liedo et al., 2013), but it is also influenced by the holding density in which adult flies were kept prior to their release. As holding densities increase, survival decreases (Gaskin et al., 2002; Hernández et al., 2010; Arredondo et al., 2015). This can explain why the values in the PVC were higher since the density of adults was minor. The survival of the adults also changed through the stages of the process. Prior to holding, values in both fly strains were significantly higher regardless of the evaluation method. In this stage flies emerged and survived only with nutritional reserves from the larval developed. Apparently, these reserves gave flies greater longevity than the food provided during holding. However, there was a 5-day-age difference among the adults evaluated in the first stage respect the other three. At the age of 5 days, males from both strains started to show some degree of sexual maturity, may be due to the food enriched protein given (see Telles-Romero et al., 2011; Orozco et al., 2013; Yuval et al., 2007; Liedo et al., 2013).

We expected that in the last three stages (pre-chilling, post-chilling and post-release), survival will not show significant differences. The main variation factor was the adult chilling process (i.e., pre and post-chilling) and this factor has proven having some side effects on flying flies but not on survival (Salvato et al., 2003; Reynold & Orchard, 2011; Andress et al., 2012; Shelly et al., 2013). For instance, studies in *B. tryioni* (Froggatt) (Reynold & Orchard, 2011) indicated that chilling does not have an effect on flies' survival after being exposed to this condition for 24 hours. In *C. capitata*, adult longevity was not affected by the chilling process at 6°C for 48 hours (Serghiou, 1977). In *A. ludens* males (bisexual and Tapachula-7 strains), survival was not affected when they were exposed for three hours to cold conditions (Arredondo et al., 2015). Survival results obtained using the PVC device concur better with this information.

Montoya et al. (2013) evaluated a very similar device to the one used in this study in the holding system for *C. capitata*. Their results indicate that it was possible to evaluate the flies' quality with the device in the different stages of the process without statistical differences respect the traditional methods, pointing out the advantages if used (i.e., consistency in the methodology among the stages of the process, evaluations under laboratory conditions). However, a difference that should be taken into consideration between the holding systems of *C. capitata* and *Anastrepha* spp. is the period of confinement. Adults of *C. capitata* are only kept for 2-days in the emergence rooms while the adults of *Anastrepha* are kept for a minimum of 4-days. Time of confinement is another factor that could influence the quality of fly adults (Zavala et al., 2010). Probably, this time difference in both fruit fly species is what makes convenient the use of the device for *C. capitata*, while in the case of *Anastrepha* species, this advantage seems not so clear. However, it is possible that with some minor adjustments, the PVC device could be used to determine these parameters in all the fruit flies

holding and release centers where SIT is applied. If the ventilation area is increased and flies density is homologated to that used in the tower's levels, then more solid and similar results could be obtained from those obtained using traditional methods. This would represent a great deal of advantages in the logistic and interpretation of results in the evaluation of these quality control parameters.

### Acknowledgements

We thank to Gladis López Rincón and Luis M. Estrada Reyes for their valuable technical assistance; also to the staff of the Mediterranean sterile flies emerging and release facility, especially to Lucy Tirado Palomeque and Moises Romero for their technical advice and assistance. To the Moscafrut Program SENASICA-SAGARPA for financial support.

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## **Field Relative Sterility Index and fried competitiveness test in bisexual and *tsl* strain from the Mediterranean fruit fly Metapa mass rearing facility**

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### **Abstract**

*Background:* The main objective of the Sterile Insect Technique (SIT) is that the mass-produced insects be as competitive as the wild insects in the field, in other words, that sterile males mate with wild females to induce sterility. Nevertheless, it has been demonstrated that in mass-rearing systems, sterile insects mating propensity is affected due to mass-rearing and sterilization processes. Since the beginning of the Mediterranean Fruit Fly Mass Rearing Metapa Facility in 1979, field evaluations of the bisexual strain and lately of the *tsl* strain have been performed, in order to assess the competitiveness of the mass reared flies in comparison to the wild flies.

*Methods:* Tests were conducted under semi-field conditions using cages 2.3 m high by 3 m in diameter using a standard protocol. To calculate the Relative Sterility Index (RSI) a ratio of 1:1:1 sterile males and wild males and females, were used for each evaluation and total mating for wild and sterile males was recorded. In the case of the Fried test, a ratio of 3:1:1, sterile males and wild males and wild females, was used.

*Results:* The RSI for both bisexual and *tsl* strains have been always above 0.2. In the case of the Fried competitiveness test there were years with less than 0.5 for the bisexual strain. Because of that, the colony was replaced by new flies collected in the wild, all of them in coffee plantations from Guatemala, since that is the area where the Program was working against the Mediterranean fruit fly. Lately this strain was replaced by a *tsl* strain which always showed RSI values above 0.5.

*Conclusions:* The *tsl* sterile males and the bisexual sterile males have been equally competitive than wild males. This was reflected both with the RSI and Fried competitiveness indices.

*Keywords:* bisexual and *tsl* strain, field cage test, mass rearing facility, mating performance, Mediterranean fruit fly, quality control.

## Introduction

The main objective of the sterile insect technique (SIT) is to massively produce sterile insects that compete in the field with wild males for wild females in order to induce sterility and decrease population levels. One key component is that sterile males are competitive and achieve matings at an acceptable level (FAO/IAEA/USDA, 2014).

It has been demonstrated that during the mass rearing process of sterile flies, irradiation affects the mating propensity of the sterile insect (Knippling, 1979; Wong et al., 1983; Lux et al., 2002). Modifications in the rearing process and inadvertent selection also affect the mating performance of the sterile insects (Liedo et al., 2007). As such, evaluation of male competitiveness becomes crucial for the success of the SIT.

The application of the Fried competitiveness and RSI tests in field cages, have allowed measuring the mating propensity and the level of induced sterility by the sterile males in competition with wild males (FAO/IAEA/USDA, 2003; McInnis et al., 1996; Cayol et al., 1999; Calcagno et al., 1999). This can give a good estimate of the effectiveness of the sterile male in the field (Liedo et al., 2007).

Since 1979, when the mass rearing facility started the production activities in Metapa de Dominguez, Chiapas, Mexico, these field evaluations with sterile flies *Ceratitis capitata* (Wiedemann), from the Metapa bisexual strain and later from the *tsl* strain, have been performed. During the first years, *C. capitata* sterile flies from the bisexual strain proved to be competitive with respect to wild males. However, in the field test performed in 1984 with the bisexual strain, a certain degree low performance was shown which ultimately led to change the laboratory colony from Costa Rica for a wild new colony from Guatemala. Later the facility started rearing a *tsl* strain.

The main purpose of this work was to show the results of the relative sterility index RSI and Fried tests that historically have been made in the Medfly program with the bisexual and the *tsl* strains. The importance of these field evaluations is discussed.

## Material and methods

a) Wild Material. Coffee berries were placed on sawdust substrate to avoid the dehydration of the collected larvae. The obtained pupae were placed in 150 mm Petri dishes and placed in Plexiglas cages with dimensions of 30x30x30 cm lined with black plastic to promote darkness. Pupa were introduced inside Plexiglas cages until adult emergence. After adult emergence, flies were separated by sex. Fifty individuals of each sex were placed in plastic bottles of two liters. Adults were fed with hydrolyzed yeast and sugar (3: 1) and water until they were 9 days old and kept under  $26 \pm 1$  °C and  $70 \pm 10$  % relative humidity conditions.

b) Sterile Material. Sterile flies came from the mass rearing Metapa de Dominguez, Chiapas facility. In the first they corresponded to the bisexual strain and since 2010 they corresponded to the *tsl* strain. In each test, males used were 7 days old. For the *tsl* strain the irradiation dose



was 125 Gy. The food provided in all evaluations was hydrolized yeast and sugar for both strains.

c) Relative Sterility Index (RSI) test. Tests were conducted in the field using cages with dimensions of 2.3 m high and 3 m in diameter (Fig.1). Fifty sterile and 50 wild males were released in the field cage. Fifteen minutes later, 50 wild females were released to obtain a ratio (1:1:1). As soon as the mating started, mating pairs were collected in glass vials. Each glass vial was then placed in a box to identify the mating type. Each trial lasted for five hours. For each day, three repetitions for a total of nine repetitions were performed.



**Fig. 1.** Field cage for evaluation of RSI and Fried test.

After each evaluation, the sterile males were identified using an epifluorescent microscope looking for the fluorescent mark in the ptilinal suture in the front of the head of the sterile insect (Fig.2).



**Fig. 2.** Identification of sterile males with epifluorescent microscope.

Competitiveness was estimated by means of the RSI (McInnis et al., 1996). The RSI is an index of the performance of mating competitiveness of sterile males, represented by the formula (FAO/IAEA/USDA, 2014).

$$RSI = SW / (SW + WW)$$

RSI values can range from 0 to 1, where 0 indicates that all wild females mated in the cage with wild males, 1 indicates that all females mated with sterile males, and 0.5 indicates that half mated with sterile and half with wild. For *C. capitata*, a mean of RSI less than 0.20 in a cage with a 1:1 S:W is a good reason to worry about the competitiveness of sterile males.

*d) Fried competitiveness test.* In order to verify that any factor that affects the competitiveness between wild and sterile males to achieve mating is reflected in the percentage of egg hatch, we performed the Fried test. For this evaluation a total of 300 sterile males, 100 wild males and 100 wild females (3:1:1) were released inside each field cage. Insects were released once and held for three consecutive days in the field cages. The substrate for female oviposition consisted of spheres prepared with agar, green dye and McCormick fucelleron which were lined with parafilm. In each field cage 20 spheres were distributed in this way, 15 on top of the cage and 5 on coffee plants that were placed inside the cages. Food (hydrolyzed protein and sugar) and water were placed in the cage. After 24 hours, the spheres were collected and replaced with new ones until the three days of assessment were completed. The collection of eggs was realized manually with dissecting forceps and placed in cups with water. In each cup, egg hatch was induced using air pumps for a period of 1 hour. Afterwards eggs were collected and placed inside Petri dishes with sponges and moist black satin and kept in an incubator at 30 °C for 72-96 hours. For each day, three repetitions were performed. Hatching

percentages as well as the Fried index were estimated. The coefficient of sexual competitiveness was estimated with Fried equation (1971):

$$C = \frac{E_s - E_c \times S}{E_c - E_e \times E}$$

$$C = \frac{E_s - E_c \times S}{E_c - E_e \times E}$$

where:

C = coefficient of competitiveness.

ES = % hatch between fertile wild flies.

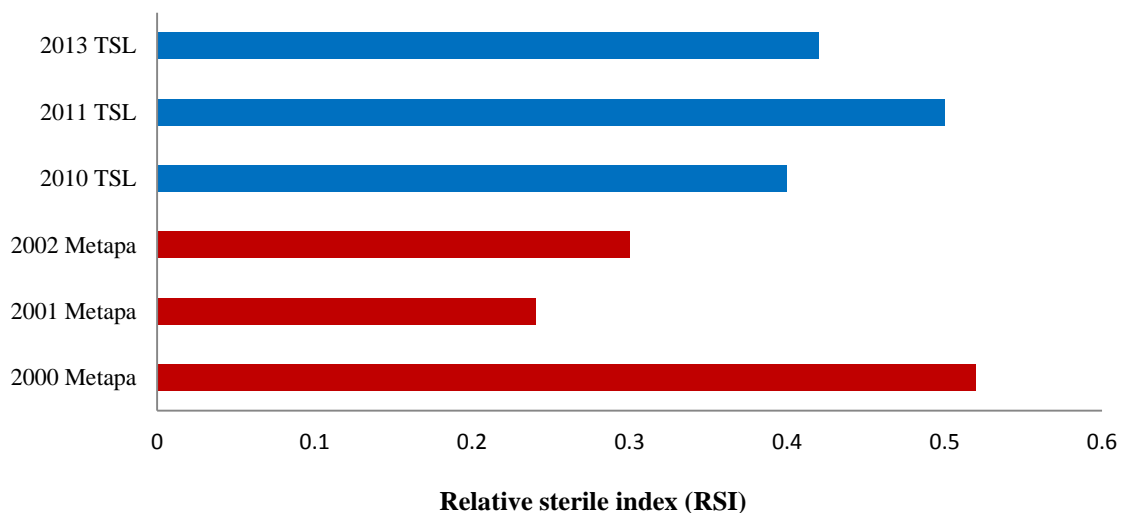
Ee = % hatch between sterile flies.

Ec = % hatch in competition.

S/E = wild: Sterile ratio

## Results

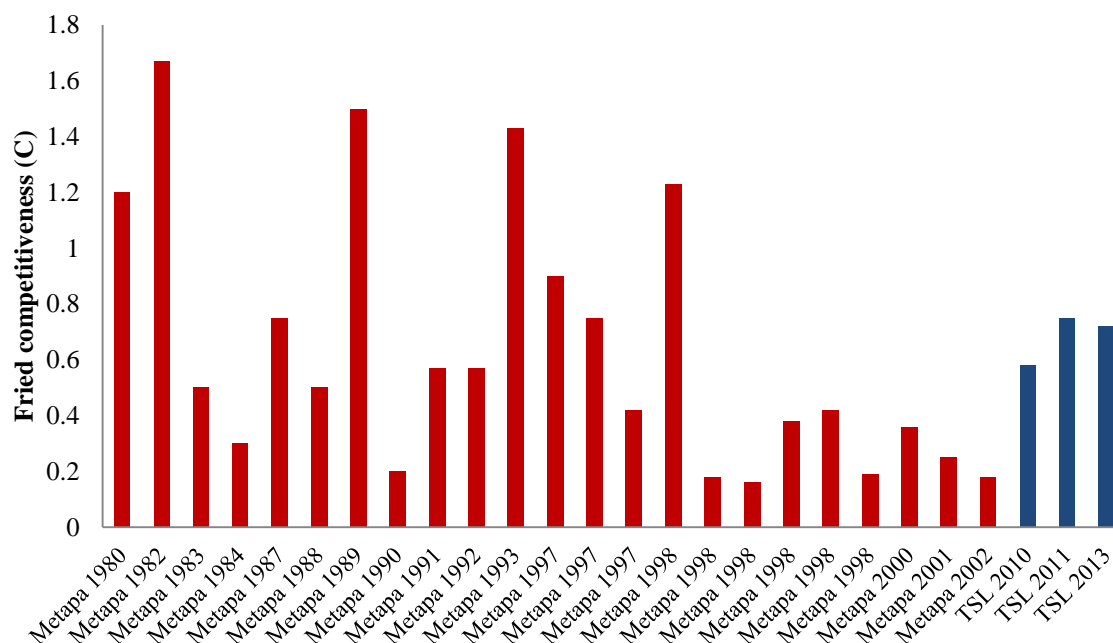
The Relative Sterility Index in both bisexual and *tsl* strains obtained showed the same trend as the results indicate that the sterile males compete with wild males to mate with wild females (Fig. 3). For the case of the *tsl* strain, the results were less variable ranging from 0.4 to 0.5.



**Fig. 3.** Relative sterile index (RSI) of bisexual (in red) and *tsl* (in blue) strains in field evaluation. Bisexual strain was evaluated from 2000 to 2002. Whereas *tsl* strain was evaluated from 2010 to 2013.

For the bisexual strain, the Fried index varied from 0.2 to 1.7. In 1998 different densities were evaluated and the densities of 1:1:3 resulted with lower values (Fig. 4).

In the case of the strain *tsl*, the results obtained were always above 0.4, indicating that the sterile males were able to compete with wild males and induce sterility in wild females.



**Fig. 4.** Fried competitiveness (C) of bisexual (in red) and *tsl* strain (in blue).

## Discussion

Field tests carried out to assess the competitiveness and induction of sterility are essential to determine the quality and efficiency of sterile flies because the results obtained allow making the estimation of how competitive are the sterile males in the field in comparison with wild males. Field tests for both bisexual and *tsl* strain were evaluated at seven days old in the case of sterile males as it is considered to be the age of maximum sexual maturity. The QC Manual recommends ages according to the species and type of fruit fly (wild-mass reared). For *C. capitata*, the age recommended for laboratory flies is 4-6 days old. In this regard, it has been reported that *C. capitata* 3 to 5 days old have sexual activity (Liedo et al., 2002).

The result with the *tsl* strain, have been satisfactory and indicates that the sterile males are able to compete with wild males. Although results are only from the Metapa facility, similar results from the El Pino facility have been obtained.

Results obtained for the *tsl* strain were relatively constant. This in addition to the elevated cost to perform these tests allow us to suggest that the tests for the *tsl* strain can be performed every other year instead of every year; alternatively they should be conducted at a given moment when changes in the mass rearing or packing systems are implemented.

In all, results from the field competitiveness field and RSI tests suggest that *C. capitata* sterile males from the bisexual and *tsl* strains mass reared and packed under the procedures of the Medfly mass rearing and packing facilities in Chiapas, Mexico, are competitive with wild males and meet the standards set by the International Atomic Energy Agency (FAO/IAEA/USDA, 2003).

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## **Sterile insect technique in area-wide integrated pest management for the establishment of a fruit fly low prevalence area in Thailand**

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### **Abstract**

*Bactrocera dorsalis*, the oriental fruit fly, was responsible for mangosteen exportation trade barrier at Trok Nong sub-district (Khlung district, Chanthaburi province), one of the tropical fruits marketable production areas of Thailand. Following great fruit losses and growers' claims, the *Trok Nong fruit flies control group* was formed in 2005, to implement environmental friendly control measures. Nearly after that, and with the technical and economic support of several institutions including Governor Office, Department of Agricultural Extension (DOAE), National Bureau of Agricultural Commodity and Food Standards (BACFS) combined with Trok Nong sub-district administrative organization (SAO) and the Thailand Institute of Nuclear Technology (TINT) an area-wide Integrated Pest Management (aw-IPM) was established. This aw-IPM pilot program was set up in a part of the Trok Nong sub-district incorporating male annihilation, orchard sanitation and *B. dorsalis* sterile insect technique (SIT). GPS and GIS were applied for area mapping, fixed sterile flies release points and trapping network system marking. A participative action plan for *B. dorsalis* control was designed by DOAE in cooperation with stakeholders. The pilot area consisted in a core area of 15.7 km<sup>2</sup>, surrounded by 10.2 km<sup>2</sup> of buffer zone. Sterile males were released at 5 million per week and per sampling site (at core area) from March to September 2013. In addition, during May 2013 an additional 5 million sterile males were released weekly both at the core and buffer areas. Results indicate that orchard sanitation, alternative and wild host removal, mass trapping and interception traps, along with sterile males' releases induce a *B. dorsalis* wild population reduction, a fruit infestation reduction (from 30% to 5%) and an increase of fruit value. Overall, results indicate a successful implementation of SIT-awIPM, and the establishment of a *B. dorsalis* low prevalence area in the Trok Nong sub-district, with a positive impact in the country. Further research is requested to fulfil the international standards of phytosanitary measures.

**Keywords:** *Bactrocera dorsalis*, control methods, low pest prevalence area, participative action plan, exportation quality.

## Introduction

The Trok Nong sub-district (Khlung district) at the Thailand's Chanthaburi province is located at 12° 27' 17" N; 102° 13' 17" E (Fig. 1). With a total area of 44 km<sup>2</sup>, of which 21.6 km<sup>2</sup> are of conserved forest and 22.4 km<sup>2</sup> for crops, is subdivided into 6 villages. In this sub-district, agriculture is the major production force, with 80% of people deserved as growers of mangosteen (*Garcinia mangostana* Linn. (Clusiaceae)), durian (*Durio zibethinus* Murray (Bombacaceae)), rambutan (*Nephelium lappaceum* Linn. (Sapindaceae)), longong, (*Lansium domesticum* Corr. (Meliaceae)), and salak (*Salacca edulis* (Arecaceae)) as major crops among others. All these major crops are the target of several species of Tephritid fruit flies, especially *Bactrocera dorsalis* (Hendel), which have been responsible of trade barrier for the same fruits and other soft fruits, especially guava that has been considered the tephritid fruit flies primary host in the region. Due to the presence of the Tephritid fruit flies and the extreme marketable quality of mangosteen produced in this sub-district, a *fruit fly control group* was established to promote and apply fruit fly control methods. Among the available control methods, the male annihilation technique followed by the Sterile Insect Technique (SIT), were applied under the umbrella of area-wide Integrated Pest Management program (aw-IPM), obtaining an area of low prevalence of fruit flies.

The present study shows how the SIT program was implemented within the aw-IPM in the Trok Nong sub-district.

## Materials and Methods

### *Study area and background*

A Trok Nong fruit fly control group was established in 2005, formed initially by a few communities and growers' leaders. This group was trained in oriental fruit fly identification and in control measures application. A participative action plan designed by the Department of Agricultural Extension (DOAE) in cooperation with stakeholders in the Trok Nong region, which jointly applied male annihilation and sterile males' releases for *B. dorsalis* control.

The Governor Office and Trok Nong sub-district administrative organization (SAO) supported, financially in 2006, the application of basic male annihilation technique to reduce *B. dorsalis* populations to levels affordable for the use of SIT. Lately in 2007, an aw-SIT program was established by the Thailand Institute of Nuclear Technology (TINT) in collaboration with Khlung district Agricultural Extension office, Burapha University, Trok Nong SAO and grower leaders, as a research project. In 2013, this program was supported by the National bureau of Agriculture Commodity and Food standards (BACFS), after that, it was supported by the DOAE.

The aw-SIT project covered 25.9 km<sup>2</sup> which included the whole cropping area (area of mangosteen, durian, rambutan, longong and salak), and some part of the conserved forest that supported 660 fruit growers households )from a total of 825 households at the Trok Nong



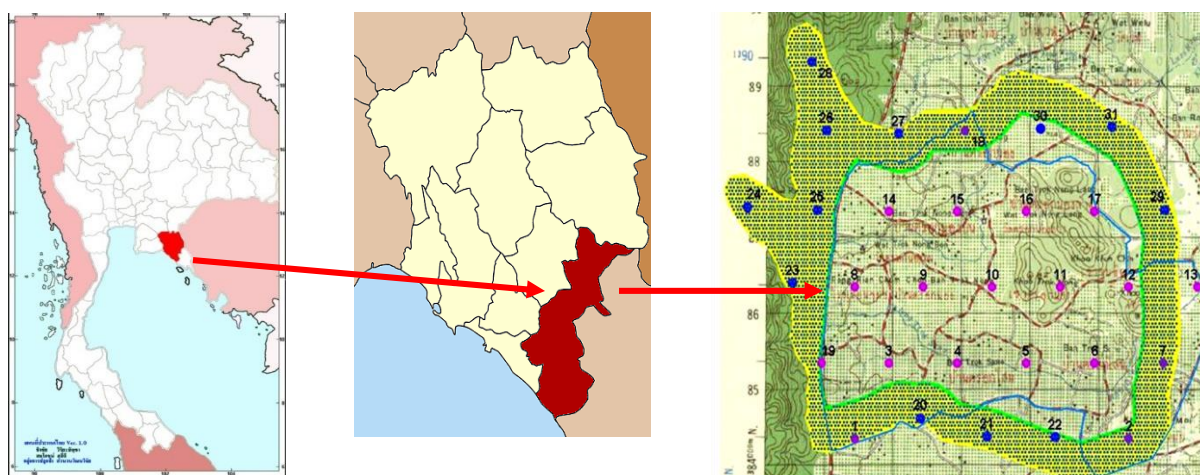
sub-district). This area comprised a 15.7 km<sup>2</sup> core area and 10.2 km<sup>2</sup> of buffer zone, established using Global positioning system (GPS) and Geographic information system (GIS) (Fig. 1).

#### *Integrated pest management program*

The aw-IPM program had required the identification of *B. dorsalis* host status of each fruit variety, after which alternative wild-hosts were removed.

Orchard sanitation was applied twice per month, in a recycle-reuse system, on which damaged or remnant fruits were composted and used as bio-fertilizer. Soil pH was measured to follow up.

Male annihilation and bait application techniques using local materials were applied for population suppression prior sterile males' releases. Traps consisted in 5 x 5 cm fiber blocks, dip-soaked in a mixture of methyl eugenol, molasses and Malathion®. These traps were applied at 50 meters interval within the core area, in a two cycles of three-month duration (Fig. 2).



**Fig. 1.** Location of the Trok Nong sub-district (in dark red) within Thailand. The red line indicate a zoom, from the Thailand country divided in provinces, then in districts, to the study area (foremost right picture) showing the distribution of the 31 monitorization traps.

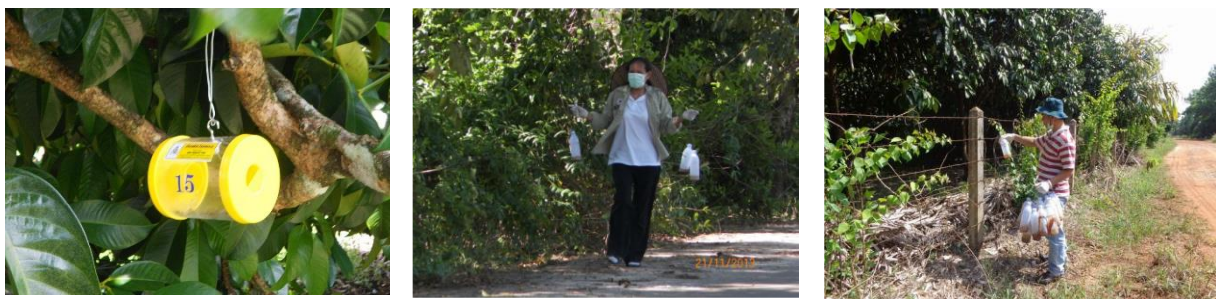
#### *Sterile males' releases and surveillance*

A surveillance/monitoring trapping network was established with modified Steiner traps distributed as 31 in the core and 10 in neighboring area as control (see first picture at Fig. 3). Modified traps were baited with a mixture of methyl eugenol, protein and Malathion®. These modified traps were applied every 25 meters interval three times a year (Fig. 3). As well as weekly quality control of sterile flies and traps service and twice a month of fruit sampling were carried out.





**Fig. 2.** Male annihilation system. Fiber blocks (5x5 cm) were soaked with a mixture of methyl eugenol, molasses and Malathion. Treated blocks were fixed on tree trunks, each 50 meters in the area core.



**Fig. 3.** Traps used to mass trapping *B. dorsalis* males and females. A modified Steiner's trap. Two growers serving liquid modified traps (150 cc of total volume baited with a mixture of methyl eugenol, protein and Malathion) fitted at 25 meters interval at the buffer area by two growers.

To avoid volunteer growers' allergic and environmental pollution caused by pupal fluorescent powder marker (as used in other SIT programs, see also FAO/IAEA/USDA, 2014), the *B. dorsalis* white-striped back strain developed by TINT was used (Boonsirichai et al., 2011). *Bactrocera dorsalis* sterile males were mass produced, being released at five millions per week rate during the following 5 years, and integrated with other control techniques as explained above. Releases were performed at ground level, by participating growers and SAO volunteers. White-striped back *B. dorsalis* strain was subjected to quality control measures (FAO/IAEA/USDA, 2014) in a weekly manner, as well as monitoring trap surveillance.

Budget and SIT technologies were supported by National Bureau of Agriculture Commodity and Food Standards (BACFS) and Department of Agricultural Extension (DOAE) in cooperating with Trok Nong SAO (Fig. 4). Growers participated with SAO response field activities for fruit flies control



**Fig. 4.** Ground release set-up for distribution of sterile *B. dorsalis* males. Sterile males' emergence cages are shown within a release facility at the district, with a zoom over a *B. dorsalis* male feeding in a leaf.

## Results and Discussion

*Bactrocera dorsalis* is a destructive fly species, native of tropical Asia, it has spread over the globe, being one of the most invasive tephritid pest species. In other countries it was also synonymised as *Bactrocera invadens* (Schutze et al., 2015), a species with a strong quarantine measures, that avoids the free-movement of fruits between infested countries, and even within countries, as the example here presented in Thailand, and the Trok Nong sub-district with its great production of mangosteen, durian, rambutan and longong. For this reason, the Thailand authorities established a participatory *B. dorsalis* control program engaging the national and regional institutions and the growers.

### *Bactrocera dorsalis* IPM: host fruits and sanitation practices

Fourteen of 18 tested fruit species were shown as *B. dorsalis* potential host, being the guava (*Psidium guajava* (L.) Kunze 1898) the preferred one. A similar result, of fruit preference for guava, was determined by Goergen et al. (2011). With these results, and taking into consideration the primary crop production at the Trok Nong sub-district, the *B. dorsalis* alternatives hosts as guava, mango, jujube and star apple trees were banned and removed by owners following the International standards for phytosanitary measures (FAO, 2012).

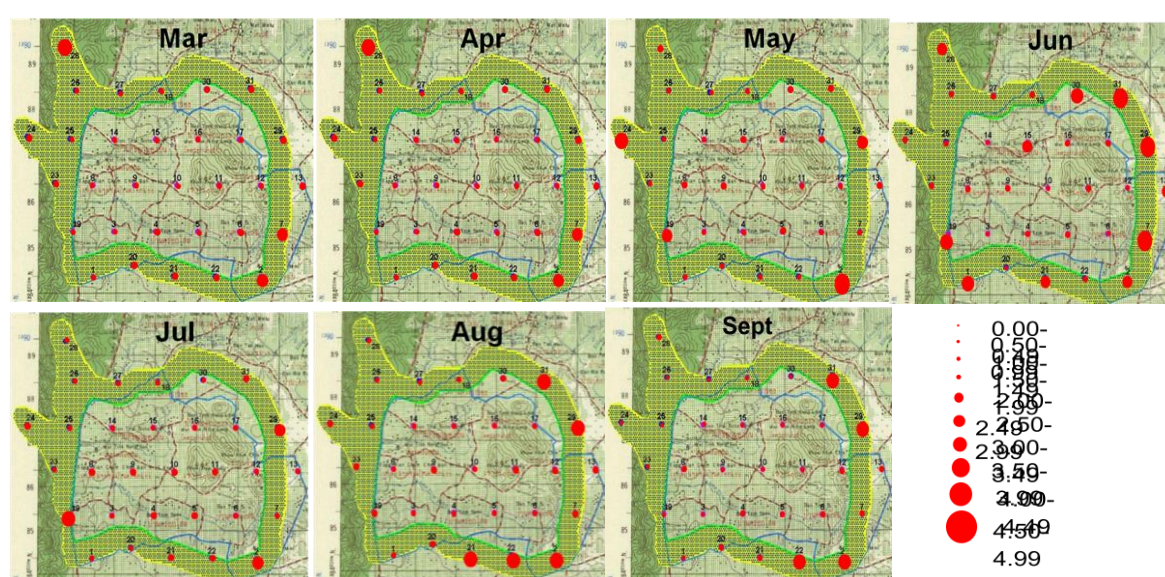
In addition to this measure, orchard sanitation also included the transformation of fall-ripen and damaged fruits into bio-fertilizers. Soil pH measure after this bio-fertilizer application indicate a raise from pH 3.0-3.5 to pH4.5-5.2, which indicate a soil quality improvement in 320 hectares. This pH raise of almost one point indicate that the soil can maintain its own



organic matter mineralization process, and that beneficial bacteria will increase its activity, both enhancing crop yield.

### *Bactrocera dorsalis* aw-SIT program

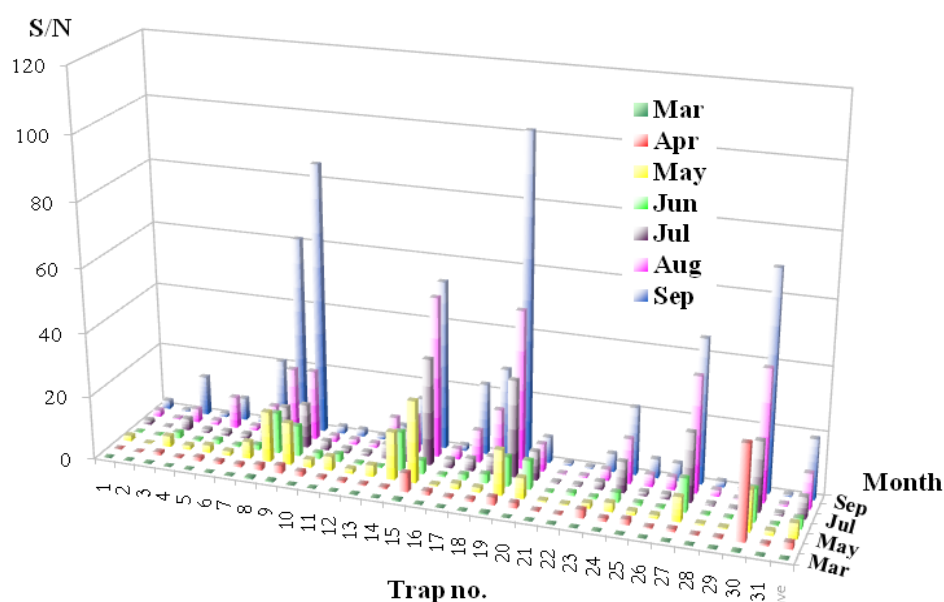
Approximately 200 millions *B. dorsalis* sterile males were released in the target area of Trok Nong sub-district (over 25.9 km<sup>2</sup>) during seven months of 2013. Monthly average *B. dorsalis* FTD (fly per trap and per day) was 0.79, 1.16, 0.82, 1.79, 1.18, 0.92 and 1.06, respectively (Fig. 5), while average *B. dorsalis* sterile/wild (or sterile/native) ratio was of 0.06, 1.17, 4.17, 2.96, 5.80, 10.08 and 16.91 (Fig. 6).



**Fig. 5.** Monthly average of FTP (flies per trap and per day) of *B. dorsalis* in Tronk Nong sub-district. The dot size indicate the scale. Each dot represents one trapping point.

The SIT-awIPM application resulted in a reduction of longong fruit damage caused by *B. dorsalis* from 30% in 2005 to 5% in 2013. Along with a reduction of chemical fertilizer cost about 406 US\$ per hectare.

At the opposite, the SIT as *B. dorsalis* control measure, resulted in increased market values, indeed, the market value of longong was increased to 83 US\$ per ton, and mangosteen to 100-167 US\$ per ton, a great increase compared to the neighboring control orchards not subjected to SIT-awIPM.



**Fig. 6.** Monthly average of *B. dorsalis* sterile/wild (S/N) ratio for each trap in Tronk Nong sub-district.

In a similar way as the Mexican SIT program (Salcedo Baca et al., 2010), the Tronk Nong sub-district SIT project is devising a high economic impact, on which fruit depreciation is being lowered due to the low prevalence of *B. dorsalis* in the region, and fruit net cost has increased allowing growers to produce high quality fruits and increase their income while reducing the number of chemical applications or production costs.

Last but not least, the *B. dorsalis* sterile:wild ratio, even if the maximum average was ~17, the single values indicated in Fig. 6, showed an increased progression with a maximum from 0 to 100 in only seven months, being an indicative of SIT success. As, when at constant sterile males release number, the ratio sterile: wild begins to increase, this is a direct measure of *B. dorsalis* wild populations' reduction.

## Conclusions and perspectives

As shown, *B. dorsalis* sterile males' releases integrated with IPM allows a significant *B. dorsalis* wild populations' reduction in the area within a short time period. Due to this success, in 2014, DOAE raised the integration of SIT within the aw-IPM program as one of its key phytosanitary measures, and more than ten thousands fruit growers in 20 Thailand provinces joined the program.

However, further efforts should be made following the International Standards for Phytosanitary Measures (FAO/IPPC, 2012) to achieve the label of *B. dorsalis* low prevalence area, under the Thailand National Plant Protection Organization certification. As even shown

an increasing high sterile:wild ratio, the FTD of wild flies is still above the 0.5 during ripening season.

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## The egg irradiation effect on genetic sexing strain of *Bactrocera dorsalis* (Hendel)

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### Abstract

**Background:** Genetic sexing strain of *Bactrocera dorsalis* (Hendel) was established for SIT application. Normally the pupae were irradiated for releasing sterile male adults. In this research we hope to explore using the irradiated eggs for rearing parasitoids of *B. dorsalis*.

**Methods:** The eggs from GSS *B. dorsalis* were irradiated with 0 Gy, 10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy, 60 Gy and 70 Gy respectively by <sup>137</sup>Cs. The egg hatch percent, mortality rate of larvae, pupation percent, pupal weight, adult emergence rate, sex-ratio (♂/♀), return mutation rate, flight ability and adult survival rate under stress were tested.

**Results:** Compared to control, the egg hatch rate was reduced with the irradiation dose increasing following  $y = 87.722 - 0.702x$ ,  $R^2 = 0.972$ . Also compared to the control, larvae mortality raised within a certain range, whereas the pupation rate decreased. Moreover, pupal weight was generally reduced and percentage of white pupa decreased respect brown pupa indicating a sex bias. The adult emergence rate decreased significantly; for male pupae the prediction equation was  $y = 92.861 - 0.377x$ ,  $R^2 = 0.943$ , whereas for female was  $y = 90.583 - 0.331x$ ,  $R^2 = 0.929$ . The adult flight ability decreased, for males the prediction equation was  $y = 82.761 - 0.519x$ ,  $R^2 = 0.948$ , whereas for females was  $y = 81.364 - 0.523x$ ,  $R^2 = 0.979$ . The adult survival rate under stress decreased significantly, especially for female adults, where the survivor rate was very low (0.33%) at a radiation dose of 70Gy.

**Conclusions:** The egg irradiation with <sup>137</sup>Cs affected significantly the development of *B. dorsalis* GSS strain.

**Keywords:** sterile insect technique, irradiation dose, egg hatch, egg development, adult performance under stress

## Introduction

The oriental fruit fly, *Bactrocera dorsalis* (Hendel), is a well-known devastating pest of citrus, loquat, guava, carambola, peach, jujube, lychee, longan and other tropical and subtropical fruits and vegetables in China (Zhu et al., 2008; Chen et al., 2011; Men et al., 2013; Qu & Sun, 2013). *Bactrocera dorsalis* causes huge losses every year in Southern and Southwestern of China (Ma et al., 2013). There were no special tactics to control *B. dorsalis*, but the sterile insect technique (SIT) as one of the important ingredients of integrated management is more and more taken seriously in China. The genetic sexing strain (GSS) of *B. dorsalis* was established and sterile males releases were applied in small scale (Ji et al., 2007; Zheng et al., 2013).

The SIT program relies on the mass-rearing and release of sterile males to compete with wild ones for mating. To achieve this mass-rearing and release, usually genetic sexing strains are developed for each Tephritid species, to reduce costs of producing irradiated females which would increase the fruit damage instead of contributing to reduce target population (reviewed in Hendrichs, 2009). But in some cases, these irradiated females are used to rear braconid parasitoids of fruit flies. A fact, that would reduce production costs while increase benefit of SIT programs. There had some studies on using irradiated hosts to rear fruit flies parasitoids in the world (Lupad 2001; Cancino et al., 2002; Cancino et al., 2009; Hendrichs, 2009), but none using irradiated *B. dorsalis* GSS as hosts.

In order to save resources while obtaining the best results, we expect to rear *B. dorsalis* GSS for both SIT programs and Biological Control programs. To achieve this objective, instead of irradiating pupae, we selected egg irradiation on *B. dorsalis* GSS strain. In this paper, we present the studies on egg irradiation effects on development and performance of genetic sexing strain of *B. dorsalis* as determined with quality control procedures from IAEA (FAO/IAEA/USDA, 2014).

## Material and Methods

### *Insects*

A GSS of *B. dorsalis* reared at the fruit fly control center in Fuzhou, Fujian, China for about 50 generations was used in these experiments. This strain was reared on artificial diet, pupated in sand and the female pupae were white, male pupae were brown. The rearing conditions were 25°C, 65±5 % RH and photoperiod of 12:12. The adult cage size was 1.2m × 0.6m × 1.2m (L × W × H).

### *Irradiation*

Eggs collected from *B. dorsalis* adults were put into 10 ml centrifuge tube, and irradiated separately at a final dose of 10, 20, 30, 40, 50, 60 or 70 Gy with a <sup>137</sup>Cs source at 1Gy per min (ie. to achieve the dose of 10Gy, the egg were exposed 10 minutes) at the Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang, China.

### *Quality tests*

Quality tests were performed as indicated in the standard IAEA procedures (FAO/IAEA/USDA 2014) which include egg hatch percentage, larvae mortality rate, pupation rate, pupal weight, sex ratio, return mutation rate, emergence rate and flight ability, and finally longevity under stress; as explained below.

#### *Egg hatch percent*

Two hundred eggs were removed from each irradiation batch, and smeared on wetted filter paper disposed in a petri dish. Eggs were allowed to develop during 72h after removal at  $25\pm 1^{\circ}\text{C}$ ,  $65\pm 5\%$  RH, and a photoperiod of 12:12 (L:D). Unhatched eggs (U) were counted after 72h, and used to determine the egg hatch percent, H%, as  $H\% = (200-U) / 200 \times 100$ .

#### *Mortality rate of larvae and pupation rate*

Two hundred larvae hatched from each irradiation dose trial were collected at random and put into dishes filled with sand as pupation substrate. After emergence counted the dead larvae B, unfinished pupated larvae C, calculated the mortality rate of larvae M and pupation rate PR.  $M\% = (B/200) \times 100\%$ ,  $PR\% = (200-B-C)/(200-B) \times 100\%$

#### *Pupal weight*

Two hundred 8-days-old female pupae (white) and 200 male pupae (brown) were collected at random separately from each irradiation dose treatment. Pupae were weighted and counted separately.

#### *Sex ratio*

When the mature larvae began to jump, from each dose treatment 200 mature larvae were collected randomly and put into dishes filled with sand at 18:00 and continued 3 days. After pupation was complete (about 5days later), pupae were counted, identified by color, white and brown, separately and calculated the sex ratio.

#### *Return mutation rate*

Three hundred white and brown pupae were randomly collected for each treatment, and kept separately in 30x30x30 cm cages for three days. After these three days, cages were frozen at  $-10^{\circ}\text{C}$  for 10 minutes. Counted the males (X1) and the total flies (Y1) from cage with white pupae; whereas females (X2) and the total flies (Y2) were counted in the brown pupae cage. The return mutation rates were calculated for white pupae (WRMR) and brown pupae (BRMR) as  $WRMR\% = X1/Y1 \times 100\%$ , and  $BRMR\% = X2/Y2 \times 100\%$ , respectively for each radiation dose.

#### *Emergency rate and flight ability*

Five black unscented plastic tubes (dia. 10cm, height 20cm ) each with 100 pupae, were placed inside an adult cage ( $1.2\text{m} \times 0.6\text{m} \times 1.2\text{m}$ , aluminum frame with 100 mesh nylon gauze). The inner walls of the plastic tubes were coated with a thin and even layer of talcum



powder to prevent flies from climbing out. To prevent the emerging flies from falling back into the tubes, 3 sticky fly strips were hung from the top of each adult cage to catch the flies. The adult cages were maintained at 25°C, 65±5% RH and a photoperiod of 12:12 (L:D). Four days later, the cage was opened and the tubes were removed for counting the following: A, number of dead pupae; B, partly emerged adults; C, deformed adults; and D, non-flying adults. Emergence rate, E%, was calculated as  $E\% = (100 - A - B) / 100 \times 100\%$ , and the flight rate, F%, as  $F\% = (100 - A - B - C - D) / 100 \times 100\%$ .

#### *Longevity under stress*

One hundred males and 100 females, within about 2 hours after emergence, were placed together in a stainless steel framed cage (30cm x 30cm x 30cm) with 100 mesh nylon gauze without water and food at 25°C, 65±5% RH under dark condition. Seventy-two hours later, the dead males (D1) and dead females (D2) were counted and used to determine the male survival rate, S1%, as  $S1\% = (100 - D1) / 100 \times 100\%$ , and female survival rate, S2%, as  $S2\% = (100 - D2) / 100 \times 100\%$ .

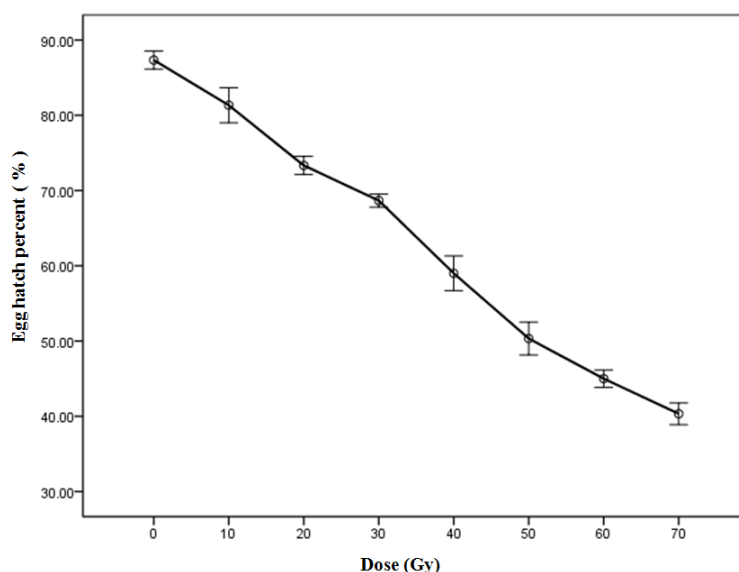
#### *Statistical analysis*

All statistical analyses were performed using the IBM SPSS Statistics 19.0 statistical analysis software.

## **Results**

#### *The influence of irradiation dose on egg hatch percent*

There existed a significant negative correlation relationship between the percentage of egg hatch and the irradiation doses, as the dose increased the egg hatch percent significantly reduced (Fig.1). When the irradiation dose was over 50 Gy, the egg hatch percent dropped to below 50%.

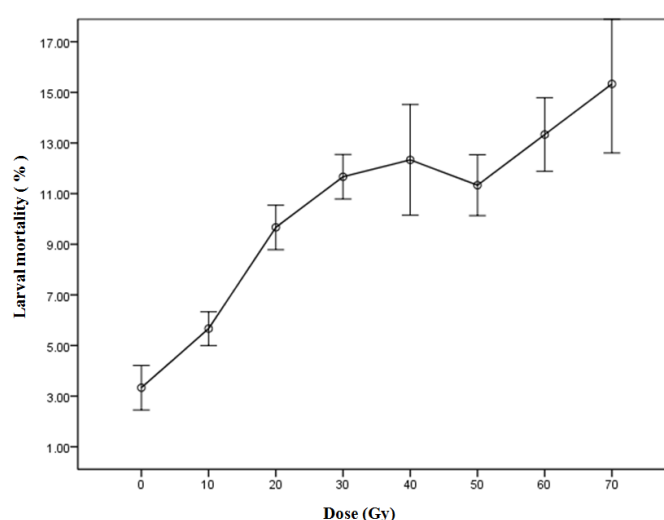


**Fig. 1.** Drop of egg hatch ability (in percentage) with radiation dose.

In linear regression analysis, the egg hatch percents and irradiation doses were in line with the equation  $y = 87.722 - 0.702x$ ,  $R^2 = 0.972$ . Linear model was tested by significant t test of the correlation coefficient r. The result showed that  $t = -27.741$ ,  $P = 0.000 < 0.01$ , and the linear regression coefficient was 0.702, which meant there was extremely significant linear relationship between egg hatch percents and irradiation doses.

#### *The influence of irradiation dose on larvae mortality*

Figure 2 showed that the larval mortalities increased with the doses increased, but when the dose was between 40 Gy and 50 Gy, the mortalities reduced and almost equal to that of 30 Gy when the dose was 50 Gy, then increased again and reached the highest i.e. about 15% when the dose was 70 Gy.



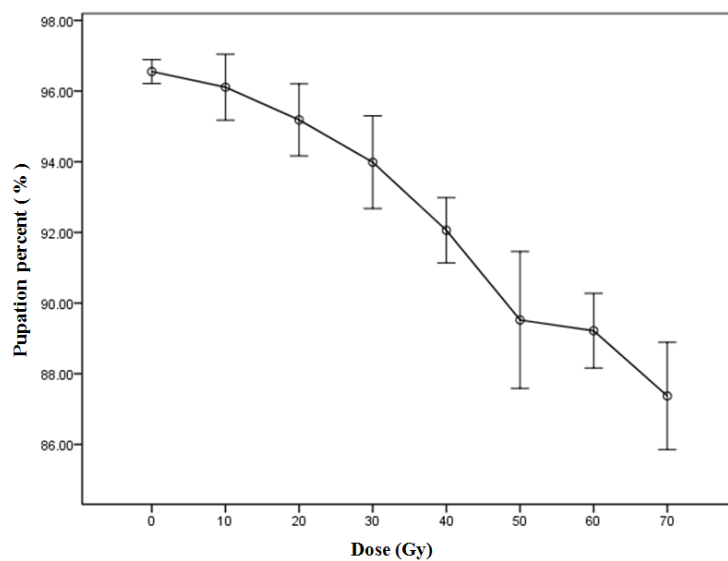
**Fig. 2.** Relationship of larva mortality (in percentage) at increasing egg irradiation dose.

#### *The influence of irradiation dose on pupation percent*

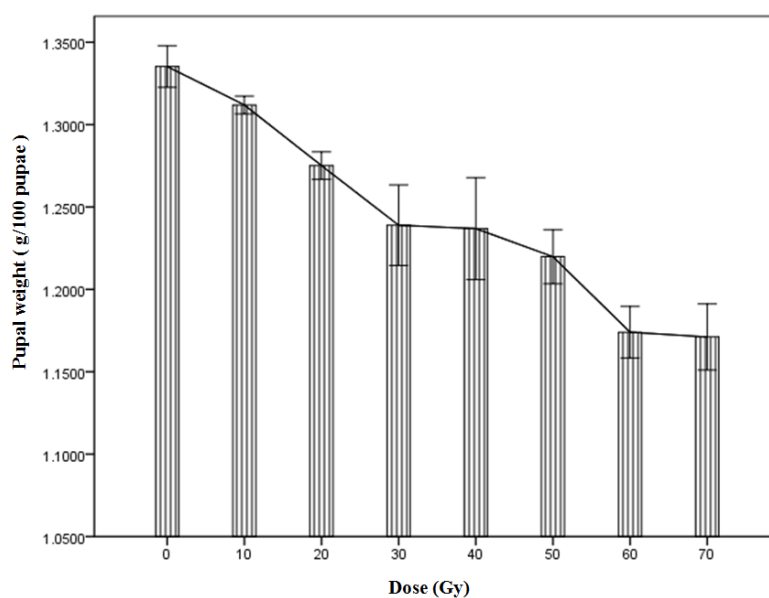
Figure 3 showed that the pupation percent of mature larvae reduced as the dose increased, but the falling trend became flat only when the dose was between 50 Gy to 60 Gy.

#### *The influence of irradiation dose on male and female pupal weight*

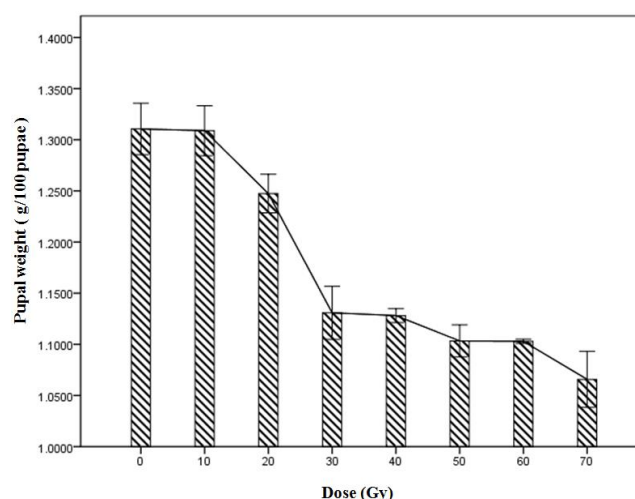
Figure 4 showed the male pupal weight decreased as the dose increased. The pupal weight decreased sharply when the dosages was from 0-30 Gy, and following smoothly when the dose was from 30-40 Gy, then decreased slowly when the dose was from 40-50 Gy, following decreased sharply when the dose was from 50-60 Gy, finally smoothly when the dose was from 60-70Gy (Fig. 4). Whereas Fig.5 showed the female pupal weights were almost stable during the dose changing from 0-10 Gy, then decreased sharply as the dose increased from 10-30 Gy, followed stable again when the dose was from 30-40 Gy, then declined steadily when the dose was from 40 -50 Gy, followed stable again when the dose was from 50-60 Gy, finally declined again when the dose was form 60-70 Gy.



**Fig. 3.** Evolution of percentage of pupation of irradiated eggs at different irradiation doses. An almost linear negative relationship is observed.



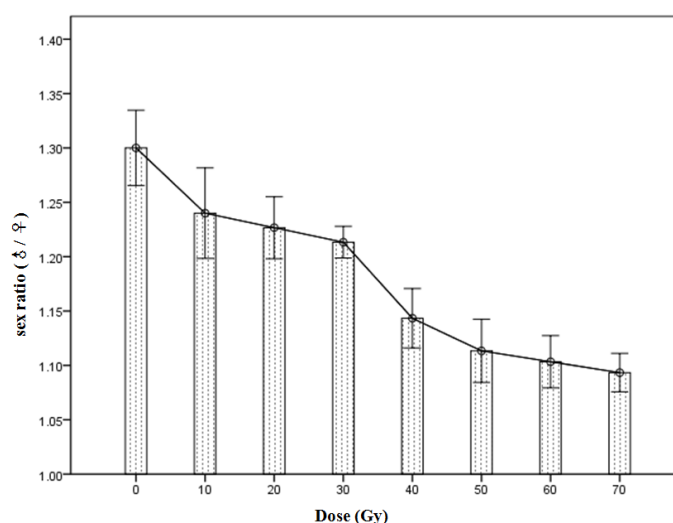
**Fig. 4.** Change regulation of weight of brown pupae with radiation dose



**Fig. 5.** Change regulation of weight of white pupae with radiation dose.

### *The influence of irradiation dose on sex ratio*

Figure 6 showed that the sex ratio (as number of males per female) declined as eggs were subjected to increased irradiation doses. When the dose was from 0-10 Gy and from 30-40 Gy the sex ratio declined sharply, the other declined stably with the dose increased.



**Fig. 6.** Change regulation of sex ratio (♂/♀) with radiation dose.

### *The influence of egg irradiation dose on return mutation rates*

Figure 7 showed that the return mutation rate of males increased with the dose increased. When the dose was from 0-30 Gy, the return mutation increased gently, but increased sharply and reached about 5.7 % when the dose was from 30-50 Gy, then increased smoothly when the dose was between 50-60 Gy, finally increased sharply and reached about 6.7 % when the dose was 70 Gy. Meaning that the GSS strain when subjected to high irradiation doses at egg

stage induce an inversion that would not allow the separation of females from the release batches.

Figure 8 showed that the return mutation rate of females increased with the dose increased and was over 4% at the dosages of 40Gy. When the dose was 60 Gy, the return mutation rate was the highest and was over 5%, then showed a little down when the dose was from 60-70 Gy.

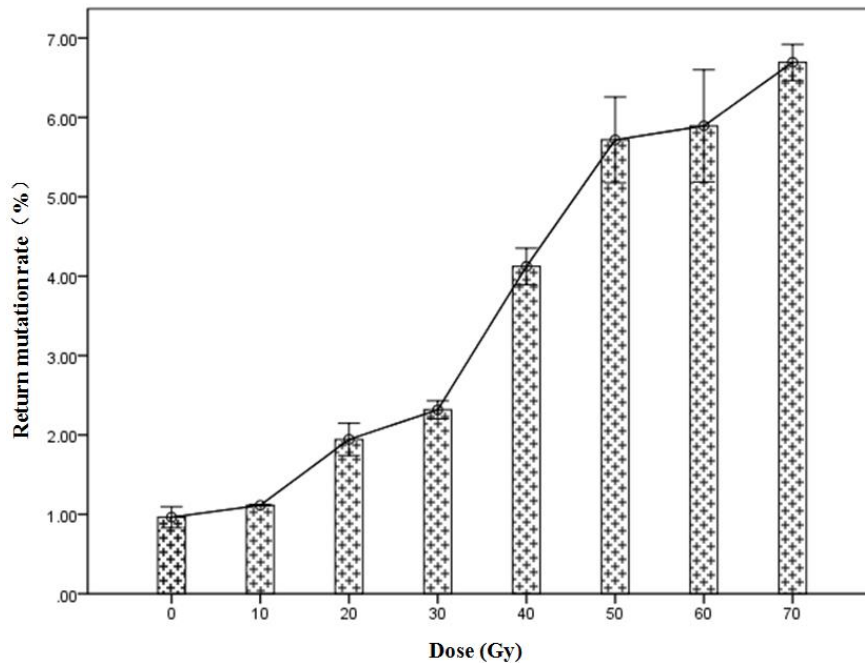


Fig. 7. Change regulation of drift rate of male with radiation dose.

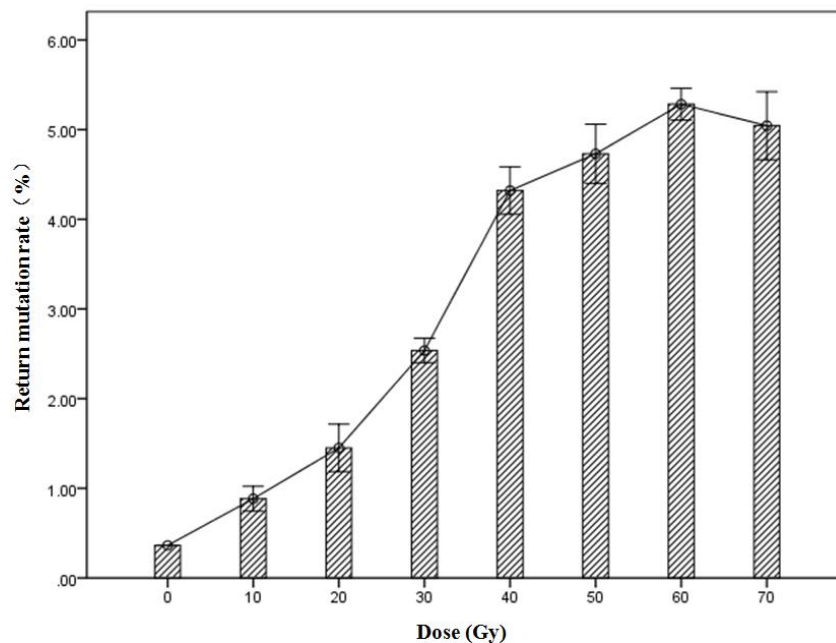
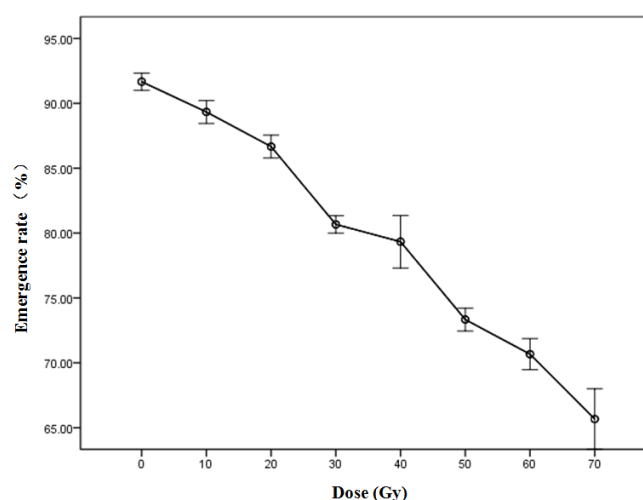


Fig. 8. Change regulation of drift rate of female with radiation dose.

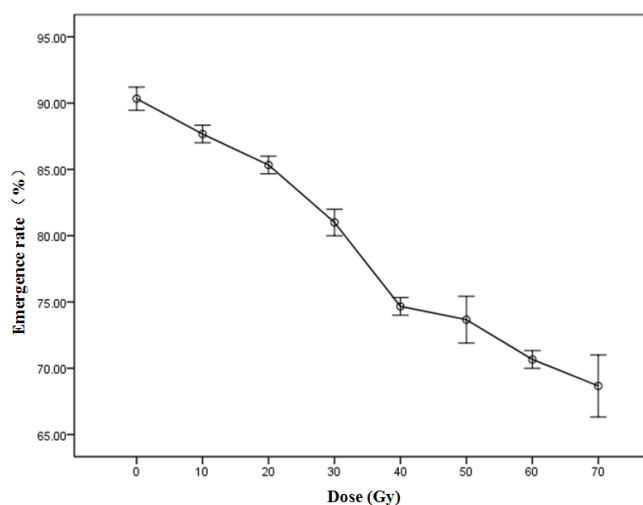
# *The influence of irradiation dose on adults' emergence rates*

The emergence rates of males decrease as the egg radiation dose increase (Fig. 9). The relationship between the male emergence rates and the irradiation dose fitted the linear regression equation:  $y = 92.861 - 0.377x$ ,  $R^2 = 0.943$ . The linear model was tested by the correlation coefficient  $r$  of significant  $t$ -test. The results showed that the  $t = -19.041$ ,  $P = 0.000 < 0.01$  and the linear regression coefficient was 0.377, meant that there existed extremely significant linear relationship between the male emergence rates and the irradiation doses.

The emergence rate of females also decrease as the egg radiation dose increase (Fig. 10). Linear analysis showed the female emergence rates and the doses fitted the linear regression equation  $y = 90.583 - 0.331x$ ,  $R^2 = 0.929$ . The linear model was tested by the correlation coefficient  $r$  of significant  $t$ -test and the results showed that  $t = -16.930$ ,  $P = 0.000 < 0.01$  and the linear regression coefficient was 0.331 (Fig. 10).



**Fig. 9.** Change regulation of emergency rate of male with radiation dose.

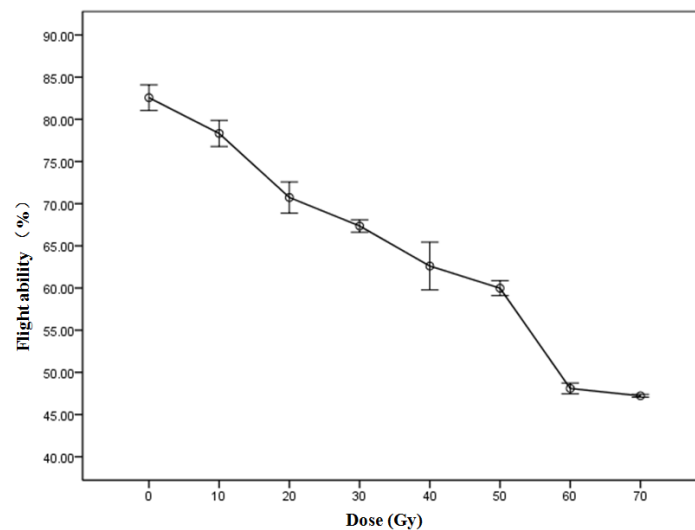


**Fig. 10.** Change regulation of emergency rate of female with radiation dose.

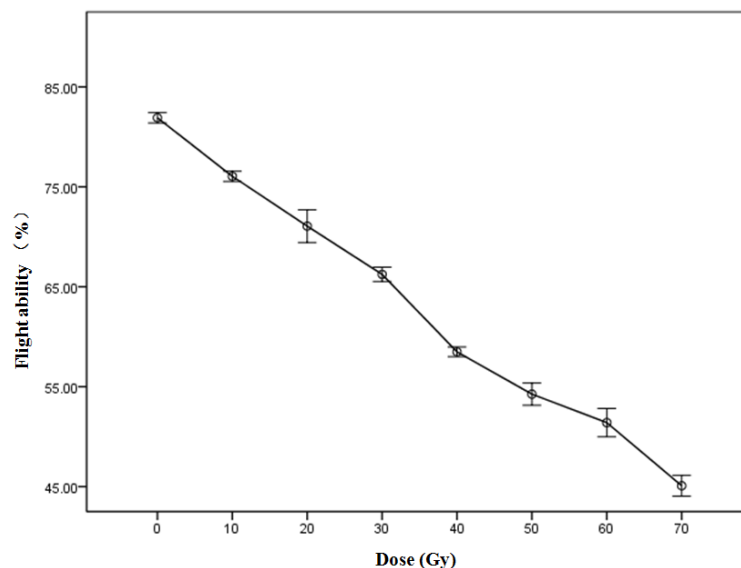
### *The influence of irradiation dose on adults' flight abilities*

The flight abilities of all studied batches decrease as egg irradiation dose increases for both males (Fig. 11) and females (Fig. 12). When the dose was 60 Gy, the males' flight ability was less than 50%. Linear analysis showed the flight abilities of male adults and the doses fitted the linear regression equation  $y = 82.761 - 0.519x$ ,  $R^2 = 0.948$ . The linear model was tested by the correlation coefficient  $r$  of significant  $t$ -test and the results showed that  $t = -19.928$ ,  $P = 0.000$  and the linear regression coefficient was 0.591 (Fig. 11).

For females, when the dose was 60 Gy, the flight ability was close to 50%. Linear analysis showed the flight abilities of female adults and the doses fitted the linear regression equation  $y = 81.364 - 0.523x$ ,  $R^2 = 0.979$ . Linear model was tested by the correlation coefficient  $r$  of significant  $t$ -test and the results showed that  $t = -32.377$ ,  $P = 0.000$ , meant there existed extreme significant linear relationship (Fig. 12).



**Fig. 11.** Decrease of flight ability (as %) of males with egg irradiation dose.

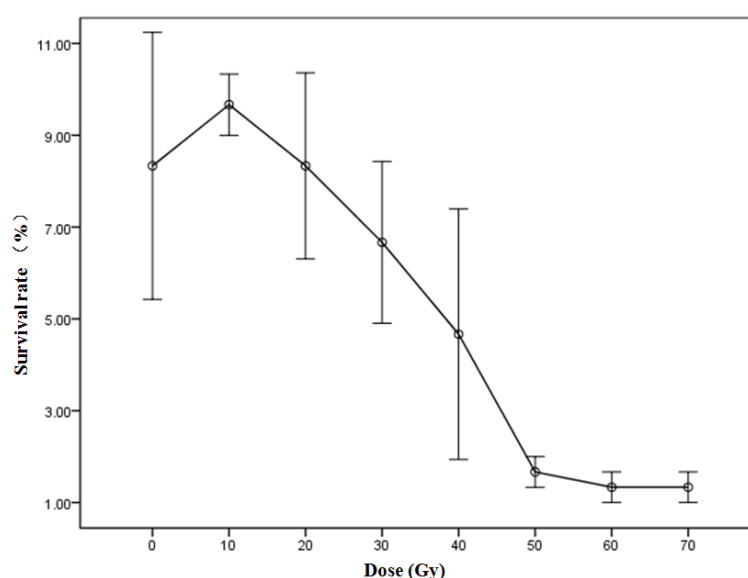


**Fig. 12.** Decrease of flight ability (as %) of females with egg irradiation dose.

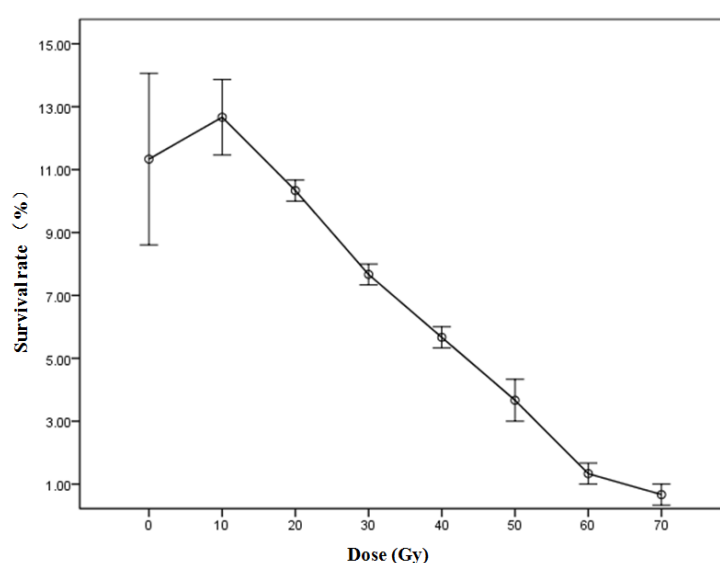
*The influence of irradiation dose on adults' survival rate under stress*

Figure 13 showed that the male survivor rate could be divided into 3 groups. The first group was from 0 - 10 Gy and the survivor rate even increased; the second group was from 10 - 50 Gy and the survivor rate dropped sharply as the dose increased; the third group was from 50 - 70 Gy and the survivor rate was very low i.e. all males almost died.

Figure 14 showed that the female survivor rate even increased when the dosage changed from 0 - 10 Gy which was similar to males, then dropped sharply as the dose increased till all flies died when the dose was 70 Gy.



**Fig. 13.** Decrease of survival rate (as %) of males with increasing egg irradiation dose.



**Fig. 14.** Decrease of survival rate (as %) of females with increasing egg irradiation dose.



## Conclusions and Perspectives

After the eggs of *B. dorsalis* were irradiated by different doses, the eggs hatch rate decreased, the mortality of larvae increased, the pupation rate decreased and the pupal weight reduced but the white (female) declined faster than the brown (male) pupae, which meant the irradiation influence on female pupal weight was greater than on the male pupal weight. The sex ratio (male/female) decreased i.e. there were more males died than females. The return mutation rates would increase caused by the irradiation at egg stage for both the males and females, and the reason maybe that the irradiation could cause the changes in the genetic structure of males for the males from GSS were sex linkage. The adult emergency rates and adult flight abilities showed significantly negative linear correlations to the irradiation doses. The survival rates under stress of adults would be as close to zero when the dose rose to 70 Gy which meant the maximum radiation dose maybe less than 70Gy if irradiation happened on egg stage of GSS of *B. dorsalis*.

In order to use irradiated eggs for mass rearing parasitoids of fruit flies, the narrow dose scope of less than 70 Gy, the parasitism rate of parasitoids on hosts and so on should be studied further.

## Acknowledgements

This work is supported by NSFC with project number 11175046.

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# **Natural Enemies & Biological Control**

## **Adaptation and first field release of *Aganaspis daci* (Weld), a larval parasitoid of the peach fruit fly *Bactrocera zonata* (Saund.), in Egypt**

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### **Abstract**

*Bactrocera zonata* has been reported as invasive species in the African continent, including Egypt, coming from the Asia neighbors. Due to the fact that is a invasive pest species, no native parasitoid was expected to be present. Indeed, the introduction of exotic parasites was a logic approach in the past against other invasive tephritidae fruit flies in other countries. Following this control measure, and in an effort to evaluate the adaptation of *A. daci* on *B. Sonata* in Egypt, a pilot trial was carried out to release and evaluate it against the pest under field conditions.

**Keywords:** biological control, exotic parasitoid import and release, invasive pest species.

### **Background and rationale**

The peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is one of the serious tephritid insect pests attacking tropical and subtropical fruits. It was first recognized in Egypt, as a new pest of guava and mango in 1998 in the northern region (El-Menshawey et al. 1999). It is now a serious pest of fruits and some vegetables replacing the Mediterranean fruit fly (Medfly) *Ceratitis capitata* (Wied.) in most of the Egyptian governorates. The exotic parasitoid species *Aganaspis daci* (Weld) (Hymenoptera: Eucoilidae) was introduced from Hawaii to Egypt through the USDA in 2008, to provide an additional mortality agent against the PFF. The native area of this parasitoid is South-East Asia, it is a larval-pupal parasitoid of several tephritid species of genus *Dacus* in Southeast Asia and Australia (Weld, 1951; Clancy et al., 1952). The parasitoid was successfully reared on *C. capitata* and *Bactrocera dorsalis* (Hendel) in Hawaii (Clausen et al., 1965). Recently, it has been reared on and released against Medfly in France, Greece and Israel (Papadopoulos & Katsoyannos, 2003).

The pest problem of *B. zonata* in Egypt is a classic example of an invasive species, moving from its native Asia to the African continent without its specific natural enemies. Therefore, the PFF invading populations increased without check and became a serious pest. It was established by the IOBC/OILB that to reduce invasive pests species to manageable levels, it was advisable to import and release candidate parasitoids from the pest's native geographic range (reviewed in Van Lenteren, 2012). Thus, the introduction of exotic parasitoids to

establish and help in suppressing the fly populations in Egypt seems a logic approach. In an effort to evaluate the adaptation of *A. daci* on *B. zonata* in Egypt, a pilot trial was carried out to release and evaluate it against the pest under field conditions.

This work presents the results of a single preliminary release trial to evaluate the parasitic potential of *A. daci* under field conditions.

## Material and Methods

### *Rearing of the parasitoid A. daci*

*Aganaspis daci* was reared on *B. zonata* at the Dept. of Biological Control, Agric. Res. Center (Giza, Egypt), as indicated previously (Hosni et al., 2011).

### *Release site and conditions*

The trial was carried out in a guava orchard, located at Al-Arish district, North Sinai Governorate (31.13° N, 33.80° E, and 0 m above the sea level), in September 2010, as this site hosts two different tephritid fruit flies, *C. capitata* and *B. zonata*, together in competition and in relatively high numbers. Twenty guava trees located in the center of the orchard were randomly chosen to release *A. daci*. Ten out of these 20 trees, were selected for sampling. The soil beneath these 10 trees was cleared from weeds and any other residues. A transparent plastic cover (2 x 3 m) was placed under each tree, and covered with a thin layer (4-5 cm) of washed sand, to be used as substrate for larval pupation. A total of 1000 adults (500 males and 500 females) were released, at a rate of 50 parasitoid adults per tree (sex ratio 1:1).

Release performance sampling was done as follows: a) a pre-release sample was taken 24 h before the release to estimate fruit fly presence; at post-release and during 6 weeks, b) fallen fruits were retrieved twice a week; c) sand-layer was checked once a week, recovering all puparia. All samples were retrieved to the laboratory, and allowed to complete development at room conditions. Parasitoid species were preserved in ethanol:glycerin (70:30, v/v) and sent for identification to the USDA (Hawaii, USA).

## Preliminary Results

### *Release trial*

The pre-release sampling indicates that a 7% and 31% of fruits were infested by *C. capitata* and *B. zonata* respectively, indicating that the release field plot was able to support *A. daci* releases, by the presence of two hosts species.

Table 1 summarizes the characterized collected pupae, as per species and within each sampling week. Data are pooled for all sampling methods.

**Table 1.** Sumarized data of pilot release trial of *A. daci* at the El-Arish region.

Sampling week	Guava fruits sampled (Kg)	Number of emerged individuals (n)				
		<i>B. zonata</i>	<i>C. capitata</i>	<i>A. daci</i>	<i>P. concolor</i>	<i>P. vindemmiae</i> + <i>D. giffardii</i>
23/09	5.2	1	8	0	0	0
29/09	11.2	0	0	6	43	5
03/10	10.8	320	82	0	7	0
08/10	7.9	153	7	0	21	13
11/10	4.8	599	6	0	24	0
18/10	3.4	491	8	0	12	0
25/10	5.8	393	1	38	42	0
10/11	2.8	615	18	6	7	0
<b>Total</b>	<b>51.9</b>	<b>2572</b>	<b>130</b>	<b>50</b>	<b>278</b>	<b>18</b>

The *A. daci* emergence peak (n= 38) was achieved from samples collected one month after release, achieving a parasitism percentage of 8%, or 1.6% for the whole surveillance period. Despite this low parasitism rate, results indicate that the released species was able to compete with naturalized parasitoids (9.7% of total emerged individuals), and that was able to parasitize either *C. capitata* (even representing less than 4.8% of emerged flies) and *B. zonata*. Indeed, when taking in consideration only the data from the best week, *A. daci* parasitism percentage (8%) was of similar percentage to the native *Psytallia concolor* (8.86%). Similarly low parasitism rates (0-17%) were observed with higher (between 5-20 times) *P. humilis* (Silvestri) (a *P. concolor* closely related species) releases on olive fields to control the olive fruit fly *Bactrocera oleae* (Rossi) (Yokoyama et al., 2010), which indicate the success of this release trial.

Moreover, taking in consideration that developmental time of *A. daci* under laboratory conditions take in average one month (Hosni et al., 2011; de Pedro et al., 2016), and that the average climatic conditions at El-Arish district in October were 28°C and 65-70% RH, resembling those at laboratory, we can conclude that released *A. daci* specimens were able to give offspring which was able at the same time to parasitize new *B. zonata* or *C. capitata* larvae already present in the guava field. But this point deserves further research, as we don't discard that the initial released parasitoids contribute also to this parasitism percentage, due to their longevity (Hosni et al., 2011), or that we have not consider the induced mortality (uneclosed pupae) that *A. daci* can exhort, which also contribute to the control of tephritid populations.

### *Identification of native parasitoids*

In addition, in this study, post-release sampling allowed to identify several parasitoid species (n=3), beside those individuals belonging to *A. daci* species, emerged from collected pupae (Table 1). Emerged parasitoid species were identified by Dr. M. Ramadan (USDA, Hawaii) as belonging to species: (i) *Psytalia concolor* (Szépligeti) (Hymenoptera: Braconidae), (n=278), a solitary larval parasitoid recorded previously in Egypt, as well as in North Africa countries and Europe, parasitizing the olive fruit fly, *B. oleae*, and occasionally on Medfly (reviewed in Wharton & Gilstrap, 1983; Daane & Johnson, 2010); (ii) *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae), an ectoparasitic idiobiont parasitoid that attacks pupae of many cyclorrhaphous Diptera, including tephritid species, and also a facultative hyperparasitoid of primary tephritid fruit fly parasitoids (Wang & Messing, 2004; Harbi et al., 2015); and (iii) *Dirhinus giffardii* Silvestri (Hymenoptera: Chalcididae), a generalist pupal ectoparasitoid attacking mainly Tephritidae species found after burrowing the pupation substrate, originally described as parasitoid of *C. capitata* in Nigeria (Silvestri, 1914 as cited in Stibick, 2004), which was recently recorded in Egypt attacking *B. zonata* (El-Husseini et al., 2008). These two last species had also been reported to act as facultative hyperparasitoids, a fact that increases concern about potential impact on newly introduced exotic parasitoids. The first species, *P. concolor*, has been also cited parasitizing medfly and other tephritid fruit flies all around the globe (see Ovruski et al., 2000).

### **Future perspectives for Biological Control of *B. zonata* in Egypt**

Despite that the first release trial indicated a putative successful naturalization of *A. daci* in the northern district of Egypt, Giza, as the parasitoid can be retrieved from naturally infested *B. zonata* or *C. capitata* guava or citrus fruits (data not shown), further studies are required to monitor and evaluate its adaptation in other Egyptian areas, along to determine its potential in suppressing pest fruit flies' populations. All these factors indicate that *A. daci* may be considered a promising species to be added to the natural parasitoid fauna already found in the region, by either inoculative or by Classic Biological control programs.

Another point that deserves further research for the successful implementation of a Biological control program of *B. zonata* based on *A. daci*, relies on the concern unveiled by the presence of other parasitoids. As indicated, *P. vindemmiae* and *D. giffardii*, can behave as facultative hyperparasitoids, which could limit the establishment of *A. daci* or at least determine the fashion on which *A. daci* releases should be performed in a future.

### **Acknowledgements**

This research was funded by the US-Egypt Joint Board on Scientific Technological Cooperation research project award IDCODE BIO11-001-014 (2008-2011).

The authors acknowledge the anonymous reviewers that improved the present manuscript.

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# **Parasitism activity of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) and *Aganaspis daci* (Weld) (Hymenoptera: Figitidae) against *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) under Mediterranean climatic conditions**

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## **Abstract**

**Background:** *Ceratitis capitata* (Wied.), the Mediterranean fruit fly, is one of the key pest species affecting citrus production around the Mediterranean coasts of Spain, Morocco and Tunisia. During the past decade the IVIA (Valencian Institute for Agricultural Research) has imported several parasitoid species to enhance a Biological Control (BC) program against this pest. Soon after the introduction of *Diachasmimorpha longicaudata* (Ashmead) in 2009, a native parasitoid, the figitid *Aganaspis daci* (Weld), was identified in Bétera, a town in the province of Valencia. This work will contribute to highlight the importance of the two species within the BC program against *C. capitata* in the study areas.

**Methods:** To determine the influence of climatic factors on parasitism rate and Medfly mortality, apples artificially infested with Medfly larvae were individually exposed to five parasitoid couples for one week under natural conditions. Forty wood-framed mesh cages (twenty for each parasitism rate and immature development) of each parasitoid species were tested weekly over 10 weeks across one year.

**Results:** Under Mediterranean climatic conditions, *D. longicaudata* exerted a high parasitism rate compared with *A. daci*. Extreme winter and summer temperatures seem to affect the immature development of both species. A higher immature mortality was observed for *A. daci* throughout the year, compared with *D. longicaudata*. Adult parasitoid species were capable of parasitizing *C. capitata* L2/L3 larvae at the extreme temperatures tested.

**Conclusions:** This study suggests that *A. daci* shows a good performance as well as *D. longicaudata* against *C. capitata* under Mediterranean climatic conditions. Further studies are required to determine the influence of other climatic factors and whether the two parasitoid species act in synergism.

**Keywords:** infested fruits, field conditions, parasitism rate.

## Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is a destructive pest of over 330 species of fruits and vegetables (Liquidó et al., 1989; Beitia et al., 2003). It is a major pest of *Citrus* and also infests deciduous fruits, such as peach, pear, and apple (Thomas et al., 2010). It originated in Tropical Africa its origin from where it has spread to the Mediterranean area and to parts of Central and South America (Malacrida et al., 2007). It is one of the most important pests of tropical, subtropical and temperate regions (FAO/IAEA, 1993). It has a great ability to disperse, to use alternative hosts and presents a great developmental plasticity which allows its survival during all seasons. In addition to crop losses, it is responsible for the establishment of quarantine restrictions that prevent or hinder the development of agricultural exports wherever it occurs (Rendon et al., 2006). In most of the countries with these problems, the control programs are essentially based on chemical treatments, mass trapping, the sterile insect technique (SIT), chemosterilization and on biological control by the use of parasitoids, despite this is the least used method (Beitia et al., 2003; Castañera et al., 2003). To establish an Integrated Pest Management program involving environmentally safe control systems (like biological control) as requested/recommended in the European normative 2009/128/CE, Spain established cooperation programs with Morocco and Tunisia for the search of native parasitoids and to share the imported ones (Harbi et al., 2015).

Nowadays in the Valencian Community (Spain), biological control against *C. capitata* is being developed and the use of two promising species is proposed: *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) and *Aganaspis daci* (Weld) (Hymenoptera: Figitidae).

*Diachasmimorpha longicaudata* is an exotic parasitoid, introduced from Mexico to Spain by the Instituto Valenciano de Investigaciones Agrarias (IVIA, Valencian Institute for Agricultural Research) in 2009 (Sabater-Muñoz et al., 2009; Harbi et al., 2015). It is a larvo-pupal endoparasitoid of fruit flies, native to the Indo-Pacific region (Oroño & Ovruski, 2007; Carbajal-Paladino et al., 2010). This is the most important parasitoid species that is being used worldwide as part of integrated pest management programs against fruit flies of the genera *Bactrocera*, *Anastrepha* and *Ceratitis* (Carbajal-Paladino et al., 2010).

*Aganaspis daci*, also from Indo-Pacific region, was found in the Valencian Community in 2009 (Sabater-Muñoz et al., 2012) in *C. capitata* pupae from fig fruits. This parasitoid species was first found exclusively in the Greek island of Chios in 2003 (Papadopoulos & Katsoyannos, 2003). This species is also a larvo-pupal endoparasitoid (Tormos et al., 2013) and is used as an agent for biological control of many species of Tephritidae (Papadopoulos & Katsoyannos, 2003).

Despite both parasitoids species are being used in many American countries, studies including abiotic factors (climatic) concerning their use in citrus crops on the Mediterranean basin (west

Palearctic eco-region) are scarce. Both parasitoids are being tested in laboratory, greenhouse, semi-field and field conditions to assess its efficacy as biological control agents of the Medfly for its inclusion in IPM programmes in citrus orchards. In this work, we show preliminary results on a semi-field experiment on the parasitism ability and induced medfly mortality of both species under natural Mediterranean conditions.

## Material and Methods

### *Experimental area*

All the trials were conducted in a 30-year old lemon (*Citrus x lemon*) plot, subjected to IPM, located in the experimental station of IVIA (Valencian Institute of Agricultural Research, Moncada, Valencia, Spain).

### *Insect rearing*

*Ceratitis capitata*, *D. longicaudata* and *A. daci*, were reared in climatic chambers at the Entomology Unit of the IVIA. The *C. capitata* 'IVIA 2002' strain was reared in artificial diet, and was used to maintain *D. longicaudata* and *A. daci* colonies, since 2009 and 2010 respectively, as described in Harbi et al. (2015). Briefly, 4-8 hours-old medfly eggs (0.3ml) were collected on water recipients located below medfly rearing cages, placed in artificial larval diet (30% Miller's wheat bran, 7.5% sugar, 3.6% brewer's yeast, and 0.24% benzoic acid, 0.2% metil-paraben and 0.2% propil-paraben as preservatives) and allowed to develop till L3 at  $25 \pm 2^\circ\text{C}$ ,  $65 \pm 10\%$  relative humidity (RH) and in complete darkness. Medfly L3 larva were either allowed to develop into adult medflies or used as host for the parasitoids. The laboratory conditions for the medfly rearing were  $27 \pm 2^\circ\text{C}$ ,  $65 \pm 10\%$  RH and 16:8 h (L:D) photoperiod; whereas the parasitoids were reared at a lower temperature  $23 \pm 2^\circ\text{C}$ , keeping the same RH and photoperiod as for the Medfly (Harbi et al., 2015; De Pedro et al., 2013). Medfly adults were feed with a mixture of sugar: yeast extract (4:1), with *ad libitum* water and house-hold granulated sugar. Adults of both parasitoids species were fed with house-hold granulated sugar, *ad libitum* water, and with a daily supply of natural bee honey (honey of thousand flowers).

### *Parasitism Experimental Protocol*

Parasitism ability was determined independently for *D. longicaudata* and *A. daci* throughout a period of twelve months (from July 2012 until May 2013) to cover all climatic seasons under Mediterranean conditions.

The experimental unit consisted in an apple (*Malus domestica* var. Royal Gala, from organic management fields) artificially infested with thirty 2<sup>nd</sup>/3<sup>rd</sup>-instar larvae of the medfly (ten holes per fruit and three larvae per hole). Apples were selected as medfly host for their availability throughout the year and by per previous results (unpublished PhD theses from A. Harbi and L. de Pedro). Each apple was confined in a ventilated plastic cylinder with five couples of the parasitoid species (3-5 days old). Each cylinder had a 50 ml water container,

and parasitoids were provided with honey every two days. Cylinders were kept inside wood-framed mesh cages as protection against rain, and placed under one single lemon tree for protection against direct sunlight. After one week of exposure, cylinders were retrieved to the laboratory, pupae collected in 125 ml plastic cups, covered with vermiculite and kept at laboratory conditions ( $25 \pm 2$  °C, 50-70% RH, 16:8 L:D) until adult emergence or up to 60 days from pick-up of pupae from the field (after this 60-days period, pupae not emerged were dissected to identify if possible, the parasitoid or medfly remains). Each trial/treatment consisted in 10 experimental units per parasitoid species, plus 10 experimental units without any parasitoid (control) to determine natural medfly mortality under each climatic condition. A total of 8 trials were conducted.

The parasitism rate was calculated for each replicate as the percentage of emerged adult parasitoids from recovered pupae (female realized fertility). The mortality of pupae (closed puparia) attributed to parasitoids was also evaluated by comparing the percentage of mortality in controls with those in treatments. This “corrected mortality” was calculated with Abbott’s formula (Abbott, 1925) as presented below.

$$\text{Corrected mortality (\%)} = ((\text{Treatment mortality} - \text{Control mortality}) / (100 - \text{Control mortality})) \times 100$$

Temperature and relative humidity were recorded by a datalogger (HD226-1, Delta Ohm, Padova, Italy) placed in one of the wooden cages with the cylinders. Other climatic conditions (wind direction, wind force, rain precipitation, UV radiation and daylight duration) were obtained from the weather station at the IVIA (located at less than 1 km from the lemon tree plot).

## Results and Discussion

The parasitism rate of *D. longicaudata* and *A. daci* and climatic conditions of each trial are shown in Fig.1. As can be observed, both species were able to parasitize larvae of medfly throughout the year, despite the differences in temperature and RH for each trial (see the differences between continuous and dashed lines in Fig.1) were noticeable.

Moreover, each species show a differentiated parasitism rates in each season (see black continuous line for *D. longicaudata* and dashed black line for *A. daci*). Indeed, it seems that each parasitoid species has its own best period, when the parasitism rate is up for one species is low for the second. Further statistical analyses of these data are required to unveil any correlation between both species.

*Diachasmimorpha longicaudata* parasitism ability on *C. capitata* larvae under natural conditions.

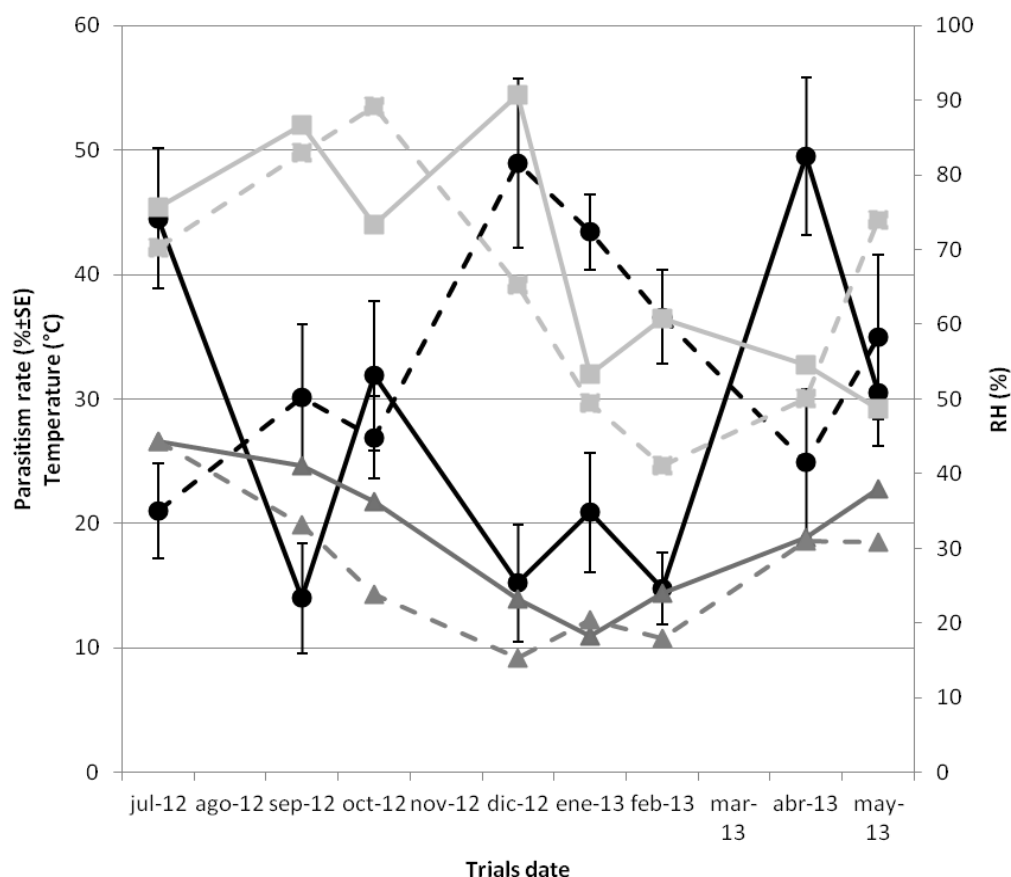
*Diachasmimorpha longicaudata* parasitized *C. capitata* larvae during all the year and the parasitism rate reached its maximum (49%) in April. The best parasitism rates were recorded in summer, autumn and spring when average temperatures were between 18°C and 26°C. In the majority of cases, the lowest parasitism rates were recorded in the coldest periods ( $\leq 15^\circ\text{C}$ ), with one exception, September 2012, with an average temperature of 22.9°C, the parasitism rate achieved was lower (14%) than the general trend. This result could be explained by the influence of other climatic factors, like precipitation (high precipitations recorded during this period, data not shown) and daylight hours (autumn-winter in northern hemisphere at Valencia latitude has an average of 6.5 h of light) that should be further examined. Under laboratory conditions Liu et al. (2012), found that the best temperature range for the development and reproduction of *D. longicaudata* reared on *C. capitata* was between 24 and 27°C. Extreme low or high temperatures had an inhibitory effect on *D. longicaudata* development. The same temperature range (24-27°C) was recorded by Lawrence et al. (1976) as suitable for the development of *D. longicaudata* reared on *Anastrepha suspensa* (Loew). Results are also consistent with those reported by Appiah et al. (2013), who found that the most suitable temperature range for the optimum parasitism rate of *D. longicaudata* reared on *Bactrocera invadens* is between 20 and 25°C. Also Sime et al. (2006) showed that this moderate range of temperatures (22–25°C) is optimal for the development of both *D. longicaudata* and *D. kraussi* reared on *Bactrocera oleae* (Gmel.).

*Aganaspis daci* parasitism ability on *C. capitata* larvae under natural conditions.

For *A. daci*, the parasitism rates were relatively uniform along the study period, with a minimum value of 21% in July, when the average temperature was the highest. In contrast, the highest parasitism rates were achieved during the coldest period, with a maximum of 48.9% in December (9.2 °C) (Fig.1).

Comparatively, *A. daci* produced a higher parasitism rate in the coldest period, when *D. longicaudata* showed the lowest parasitism rate values. Given that no published data about the effect of the temperature on the development of *A. daci* is available, our results suggests that this figitid species may be adapted to lower temperatures compared to *D. longicaudata* (Fig.1). In the opposite hand, at average temperatures higher to 18°C, the parasitism rates of *D. longicaudata* were better than those of *A. daci* (Fig.1).

The results show the absence of a direct relationship between the temperature and the rate of parasitism of both species, suggesting that other climatic factors can play a major role on it, which deserve further analyses.



**Fig. 1.** Parasitism rate (%  $\pm$  SE; in black), average temperature (Celsius degrees; in dark grey) and relative humidity (%; in light grey) obtained for each trial and parasitoid species. Continuous lines for *D. longicaudata*, and dashed ones for *A. daci*.

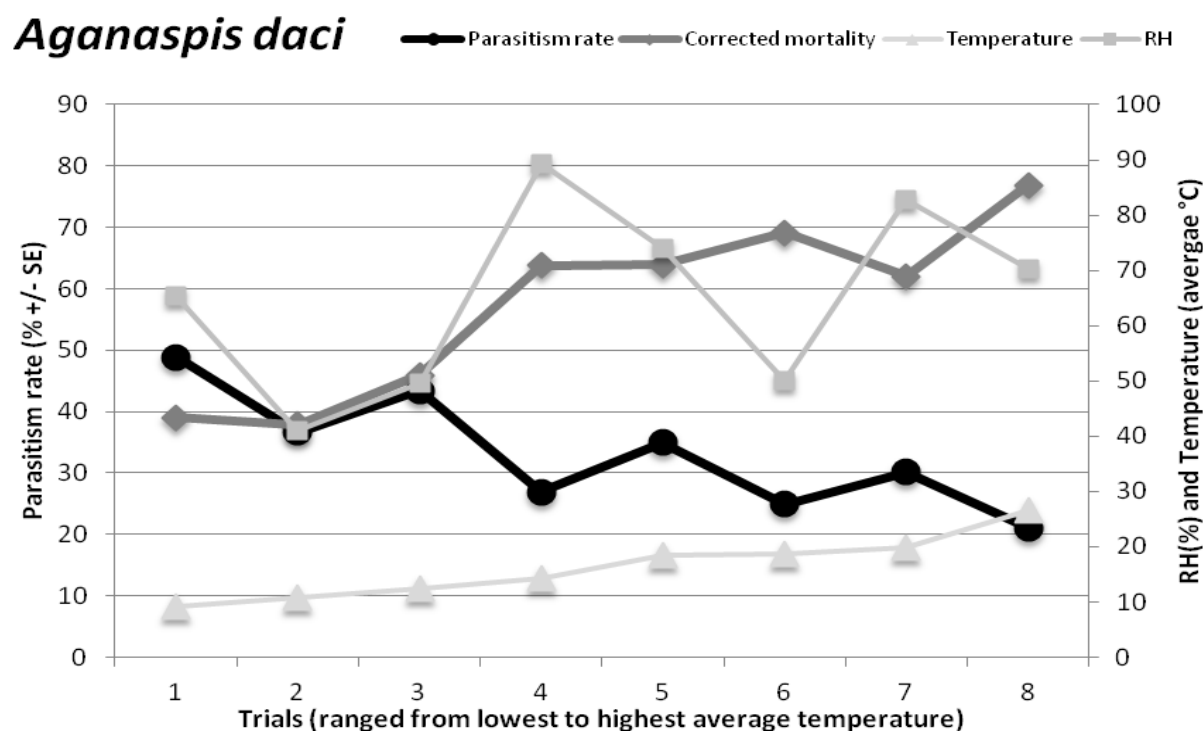
#### *Parasitoid effect on C. capitata* mortality

Corrected mortality, following Abbott's formula, puts into value the effect of each parasitoid, as includes the mortality of parasitoid immature stages (due to unfavorable climatic conditions) and medfly pupae killed by parasitoid females (by multiple oviposition events without the presence of parasitoid egg). Table 3 summarizes the results of mortality accounted in *C. capitata* pupae (denoted as pupa not emerged) exposed to *D. longicaudata* and *A. daci*, with the corresponding natural mortality of the controls.

In general, *C. capitata* mortality induced by *A. daci* was higher to that induced by *D. longicaudata* throughout the year (Table 1). The contribution of abiotic factors (climatic conditions) to this induced mortality should be further studied. However, as can be observed in Fig.2 and Fig.3, it seems exists a relationship between mortality, average temperature and RH, in opposite directions for each parasitoid species. As can be observed *A. daci* induces high mortality during hottest periods whereas *D. longicaudata* does it in coldest periods (Fig.2 and Fig.3 respectively).

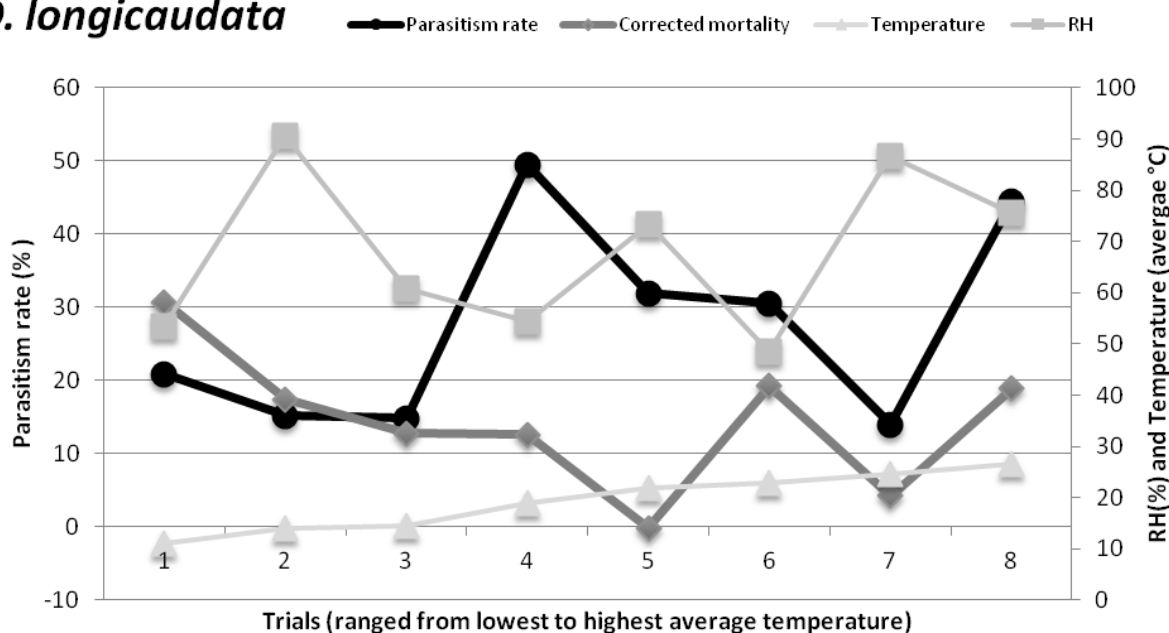
**Table 1.** *Ceratitis capitata* immatures mortality (expressed as %) in treatments, corresponding controls, and the corrected mortality (Abbott's formula) due to the parasitoids *D. longicaudata* and *A. daci*.

Period	<i>D. longicaudata</i>			<i>A. daci</i>		
	Treatment	Control	Corrected	Treatment	Control	Corrected
July 2012	19.9	1.1	18.9	78.9	8.8	76.9
September 2012	15.8	12.1	4.2	64.3	6.1	62.0
October 2012	8.7	8.9	-0.2	67.2	9.3	63.8
December 2012	19.4	2.4	17.4	45.9	11.3	39.0
January 2013	37.4	9.7	30.7	52.9	13.0	45.9
February 2013	16.9	4.6	12.8	39.3	2.3	37.9
April 2013	13.6	1.1	12.6	72.0	9.4	69.1
May 2013	24.6	6.7	19.2	67.6	10.3	63.9



**Fig. 2.** Evolution of *A. daci* parasitism rate (% , black line) and corrected mortality (% , dark grey line) over *C. capitata* along the experiment, when trials are arranged in average ambience temperature (triangles in light grey) ascending order.



***D. longicaudata***

**Fig. 3.** Evolution of *D. longicaudata* parasitism rate (% , black line) and corrected mortality (% , dark grey line) over *C. capitata* along the experiment, when trials are arranged in average ambience temperature (triangles in light grey) ascending order.

It is important to note that, in general, the mortality induced for *A. daci* on pupae was higher compared to that induced by *D. longicaudata* throughout the year (Fig.2 line in dark grey vs Fig.3 in dark grey). It can be suggested that the corrected mortality reflects mainly the mortality of immature stages of the parasitoids: high rates of parasitism is related with low rates of mortality, so this fact reinforces the idea that low temperatures favor the parasitism of *A. daci* and in opposite side, *D. longicaudata* seems to prefer higher temperatures.

In summary, the results show that the reduction of medfly populations by the two parasitoid species can be affected not only by the parasitism activity but also by the mortality induced on pupae, and that both pest control factors are affected by climatic conditions (temperature and RH), but this last step deserves further research. In general, both species can be considered as potential biocontrol agents against the medfly in the Valencian Community, and that these results can be translated to Tunisia in the Mediterranean Region, which shows nearly similar climatic conditions. However, more work must be done to determine the way these parasitoids may interact in the field and along the time. Studies on the influence of the temperature and other climatic conditions (as the photoperiod, RH and the rainfall) on the parasitism rate of each of these two species, and also to know the effect of those climatic conditions on the development and mortality of the pre-imaginal stages of both parasitoid species in the field will surely provide valuable information.

## Acknowledgements

The authors thank the collaboration of A. Duato and M.J. Camaró in the maintenance of the insect colonies. We also thank Dr. Devescovi and to the anonymous reviewers for helpful discussion and improvement of this manuscript. This work has been developed within the framework of the research project of the Ministry of Economy and Competitiveness AGL2010-21349-C02-02. The participation of F. Ferrara has been possible thanks to a grant of the CNPq from Brazil and A. Harbi has benefited from a scholarship of the Ministry of Education in Tunisia.

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# **Risk Analysis, Quarantine & Post Harvest**

## **Pest risk analysis for economically important Tephritidae: The crossroads between science, plant protection, and safe trade**

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### **Abstract**

*Background:* Some of the most important pests that stand in the way of safe agricultural trade belong to the family Tephritidae. As agricultural trade continues to increase, the risk that fruit fly and other pests will spread into areas where they do not occur also increases. However, at the same time, the budget that many Federal and State governments have for preventing and detecting pest outbreaks is shrinking. Therefore, prioritization of potential pest threats and how to mitigate them is becoming increasingly essential.

*Methods:* Pest Risk Analysis (PRA) is an essential tool that helps countries proactively protect their plant resources from the risks posed by pests. It begins by collecting and evaluating scientific and other information to assess the likelihood that pests will be introduced into new areas, and then estimating the consequences that would result from these introductions. Based on the results of the assessment, recommendations can be made for mitigating the risks. This process allows countries to make regulatory decisions that are better informed, defensible, and rational, and to prioritize and direct their resources towards the pests that pose the greatest risk. In this presentation we will discuss the kinds of scientific information crucial to accurately assess the risks posed by tephritid fruit flies moving in international trade and the role that a closer association between researchers and regulators can have in understanding the context of new entomological knowledge. The second part of this presentation will discuss the development and application of bio-economic models and their usefulness to accurately assessing the risks from tephritid introductions.

*Results & Conclusions:* Pest risk analysis is an evolving and dynamic field. In the past 20 years, PRA has undergone an evolution - pest risk analysts have adapted methods used in other sectors, scientific information that supports analyses is more available than ever, and the need for more, better and different types of pest risk analyses continues to grow. Moreover, regulatory decisions affecting imported products are now required under international treaties to be supported by an assessment of risk. However, predicting how pests will behave in novel environments is always difficult and contains large amounts of uncertainty. In many cases, the information we rely on to conduct a PRA may not be fully accurate or up-to-date. In some cases the information may be incomplete, misleading or absent. Nevertheless, regulators have to make decisions in the absence of perfect information. Because of this, it is essential that the research and regulatory communities work closely together. Researchers need to understand

the kinds of information needed for better PRA, and regulators need to have access to the best and most reliable information.

*Keywords:* bio-economic modeling, pest prioritization, pest risk analysis, safe trade, Tephritidae.

## **Introduction**

For most countries in the world, international trade and, in particular, the import and export of agricultural commodities has been essential to maintaining and improving the health of their economies and their citizens. As agricultural trade has increased and trade barriers have been removed, importing and exporting countries have enjoyed the benefits of world commerce, including greater wealth and economic growth as well as improved availability of competitively priced plants and plant products, which has had positive impacts on food security and the well-being of a country's inhabitants.

However, more trade, faster trade, and the opening of new markets for agricultural commodities also has brought greater opportunities for the introduction and spread of pests that can have deleterious consequences to a country's managed as well as natural ecosystems. Some of the most important pests that stand in the way of safe agricultural trade belong to the family Tephritidae – the true fruit flies. According to White and Elson-Harris (1992), approximately 250 species of Tephritidae are known to be associated with commercially-produced fruits and vegetables, and about 70 of these species are significant agricultural pests. Their inconspicuous eggs and early instar larvae and their multivoltine life histories, combined with relatively long adult life stages and highly fecund females, greatly increase the likelihood of establishment and spread of these pests when following the pathway of agricultural commodities traded internationally.

## **International Regulatory Framework for Safe Trade**

It is the sovereign right of all countries to protect their human, animal, and plant life and health. In the context of international trade, this sovereign right includes requiring measures to ensure that imported food is safe for consumers and imported animals and plants do not bring with them harmful pests and diseases. These measures are referred to as “sanitary (human and animal health) and phytosanitary (plant health) measures” or simply “SPS measures.” SPS measures can take many forms. A few examples include: prohibiting the entry of commodities known to carry pests; requiring that plants or animals intended for import come from disease-free areas; inspecting or testing food products for contaminants; and mandating specific post-harvest treatments for products (WTO, 2007).

In order to minimize the risks posed to plant life and health, importing countries may require that phytosanitary measures be in place before agricultural commodities are allowed to enter that country. However, if these measures are *too* stringent, they will cancel-out the benefits gained from allowing trade, thus mimicking the detrimental effects of traditional trade

barriers (e.g., tariffs, subsidies, quotas) (Henson & Loader, 2001; Costello & McAusland, 2003; Jaffee & Henson, 2005). For this reason, it is essential that countries accurately identify and characterize the risks associated with international trade of plants, animals and foodstuffs before SPS measures are identified.

During the Uruguay Round of multilateral trade negotiations and the creation of the World Trade Organization (WTO), the agricultural sector expressed concern that SPS measures were being disguised as substitutes to traditional trade barriers in order to restrict foreign competition and distort trade. They feared that as more tariffs and other trade barriers were removed, such “protectionist” SPS measures would increase (Jaffee & Henson, 2005; WTO, 2005). The WTO’s Agreement on the Application of Sanitary and Phytosanitary Measures, or SPS Agreement, addressed these concerns by providing a legally binding framework to ensure that SPS measures are applied *only* to the extent necessary to protect (human, animal and plant) life and health and that these measures are *technically justified* (Devorshak, 2007).

Hence, the two main objectives of the SPS Agreement are: 1) to protect and improve human, plant, and animal life and health for all member countries; and 2) to protect all member countries from arbitrary or unjustifiable discrimination due to requirement of unnecessary sanitary and phytosanitary measures (Henson & Loader, 2001; WTO, 1994; Devorshak, 2007). The SPS Agreement guards the sovereign right of countries to choose their own “appropriate level of (sanitary or phytosanitary) protection,” as long as the SPS measures to achieve that level of protection are technically justified and are the same for domestic producers and foreign trading partners. Importantly, however, the SPS Agreement also requires members to “take into account the objective of minimizing trade effects” (APHIS, 1998; Henson & Loader, 2001; Jaffee & Henson, 2005; Devorshak, 2007).

According to the SPS Agreement, countries concerned about protecting their plant life and health must justify their phytosanitary measures with an assessment of the phytosanitary risks that takes into account:

- Scientific evidence
- Relevant processes and production methods
- Prevalence of pests and diseases
- Existence of pest- or disease- free areas
- Relevant ecological and environmental conditions
- Quarantine and other treatments
- Potential damage due to loss of production or sales in the event of pest or disease introduction and spread
- Cost of control or eradication of a pest or disease
- Relative cost effectiveness of alternative approaches to limiting risks

## What is Pest Risk Analysis?

The International Plant Protection Convention (IPPC), which is the standard setting organization for plant health named in the SPS Agreement, defines Pest Risk Analysis (PRA) as “the process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it” (FAO, 2007).

As such, a PRA document provides the technical justification for phytosanitary measures required to prevent the introduction (entry and establishment) and spread of pests and diseases. Pest Risk Analysis has three components: risk assessment, risk management and risk communication.

During the conduct of risk assessment for a newly proposed internationally traded agricultural commodity, potentially harmful pests and diseases associated with that commodity in the exporting country are identified. If these pests or diseases are found to have the *potential* of causing economic harm, *are not present* in the importing country *or* are present, but *have a limited distribution and are under official control* by the National Plant Protection Organization of the importing country, then these pests or diseases meet the IPPCs definition of quarantine pest. Once quarantine pests are identified, the risk assessment characterizes the risk(s) posed by each quarantine pest by estimating the likelihood (or probability) that each pest will be introduced (enter and establish) into the importing country. The risk assessment continues by determining the consequences of introduction of each quarantine pest.

During risk management, the second component of a PRA, options for mitigating or managing the risk(s) are sought. These phytosanitary (or risk management) measures should have a rational relationship with the risk(s) identified in the risk assessment. The options are evaluated for their efficacy, feasibility, and impacts on the pest risk(s). Risk management concludes with a recommendation on the options that are best suited for the situation.

Risk communication, the final component of PRA, occurs throughout the PRA process. In fact, the PRA document itself is a form of risk communication. Risk communication is a two-way process, essential throughout the development of the PRA, as all parties must be able to effectively explain how they understand the identified risks and how they can be reduced. For example, in risk assessment, communication with stakeholders is essential to correctly identify and characterize all potential risks and understand the potential consequences. Additionally, in order for stakeholders to have a realistic understanding of the risk(s), the results of the risk assessment need to be clearly communicated to them. Furthermore, risk management will not be effective unless there is good communication with those who assesses the risk, as well as with anyone who might be impacted by risk management decisions.



## Elements of Risk Assessment

*Risk* is defined as the likelihood (or probability) of an adverse event occurring and the magnitude of the associated consequences. In the protection of plant health and in the context of international trade, the adverse event we are concerned with is the introduction and spread of quarantine pests. The likelihood that a pest will be introduced into a new area depends on a series of events, each of which has some probability or likelihood of occurring. When analyzing the likelihood of introduction of a quarantine pest, it is important to identify all of the events that might play a role in effecting this introduction. This series of events is referred to in some countries as a “pathway of introduction”. If any one of these events has a zero likelihood of occurring - for example, if the pest is not going to remain with the commodity through harvest and packaging - the overall likelihood of introduction will be zero.

Evaluation of whether a quarantine pest follows the pathway is a complex process. Scientific and other evidence is gathered and used to inform the analysis. Factors that influence the likelihood that a particular pest will be introduced include traits of the plant or plant product being imported, pest biology and behavior, and industry practices and their effects of pest prevalence. The conclusion of the analysis is a judgment made by the risk analyst, colored by their experience and supported by the available evidence.

The associated consequences, the second component of the risk equation, are the possible impacts that will result from the introduction of the quarantine pest into the area being analyzed (this area could be equal to the entire importing country or portions of that country where conditions are favorable for pest establishment). Impacts can be direct (those that occur as a result of the adverse event being realized) as well as indirect (those that occur as a consequence of the direct impacts). Consequences (or impacts) are additive; in other words, the sum of each of the consequences equals the total impact of the adverse event. However, it is not always necessary to identify all of the potential consequences of introduction of a quarantine pest. Often, it is sufficient to show that the combination of consequences (or impacts) will be greater than a certain amount - in other words, that the consequences will exceed the appropriate level of protection that the importing country would allow.

Potential consequences that may result from the introduction of a quarantine pest may include:

- Damage to hosts (that would not have occurred if the pest had not been introduced)
  - Yield Loss
  - Reduced population
- Displacement of other species
- *Additional* costs of control
- Effect on existing production practices
- Loss of markets
- Changes to trade
- Changes in consumer demand

- Costs of restoring environment
- Impacts on other industries (e.g. tourism, energy)
- Social effect of additional control measures
- Changes to plant communities
- Reduction in aesthetic beauty
- Impacts that occur as a result of pest effects on other organisms or hosts

Based on the results of the risk assessment, a decision must be made about whether the risk is acceptable. If not, risk management analysis begins.

Factors that influence the decision of whether or not a given SPS measure should be required include: feasibility of the SPS measure, biology of the pest, the area over which the measure may occur, and the demand for and relative benefits of undertaking such a measure (Devorshak, 2007). In selecting optimal SPS measures, plant protection officials should consider which option minimizes the damages and control costs of the pest over time (accounting for the possibility of reoccurrence) while also minimizing the social costs associated with each strategy (Olsen, 2006).

### **Information needs when conducting Pest Risk Analysis**

Predicting how quarantine pests, including tephritid fruit flies, will behave when they encounter new environments is difficult and contains large amounts of uncertainty. In many cases, the evidence and information needed to prepare a PRA may be unavailable, inaccurate, incomplete or outdated. In some cases the information may be misleading. Nevertheless, in order to protect plant life and health, risk analysts and regulatory policy makers are forced to make judgments and regulatory decisions in the absence of perfect information, attempting to accurately predict an event that has not yet occurred and trying to identify ways to manage it. Because of this, it is essential that the research and regulatory communities work closely together to ensure that the best evidence is available to inform a PRA and make appropriate regulatory decisions. It is equally important for researchers to understand the kinds of evidence and information needed to inform a PRA and for risk analysts and regulatory policy makers to have ready access to the latest and highest quality scientific and other evidence.

In order to assess the risks posed by fruit flies moving in international trade, risk analysts rely on a variety of scientific and other information including:

- Taxonomy
- Hosts
- Distribution
- Biology – life cycle, reproductive, survival, dispersal strategies, hosts
- Environmental requirements and constraints
- Type and magnitude of damage
- Available management practices

Estimating the likelihood that a quarantine significant fruit fly will be introduced, and predicting its consequences of introduction can be very difficult even under the best of circumstances. The complexity of ecological systems makes it difficult to predict how a new pest will behave in a new environment. Furthermore, natural variability in pest populations means that not every individual will behave in the same way. By working together, researchers and plant protection officials can reduce the uncertainty in PRA leading to better, more informed regulatory decisions.

## Conclusion

PRA evaluates biological or other scientific and economic evidence to determine whether an organism is a pest and whether it should be regulated, and assists in determining the strength of phytosanitary measures to be taken against it. As such, PRA facilitates safe and fair trade in plant and plant products and is an essential tool that helps countries proactively protect their plant resources from the risks posed by pests. PRA allows countries to make regulatory decisions that are better informed, defensible, and rational.

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## USDA Compendium of fruit fly host information (CoFFHI)

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### Abstract

**Background:** Tephritid fruit flies are serious agricultural pests. Knowledge of the host status of different fruits and vegetables is needed in support of the development of systems approaches to facilitate commodity exports as well as to readily permit quarantine regulatory officials to assess the risk of introduction of these fruit fly species in imported or exported fruits and vegetables. To provide this needed knowledge, we began development of an interactive compendium of the host plants of economically significant fruit fly species.

**Methods:** The host plant database is being developed using two complementary strategies: one, developing comprehensive, online accessible fruit-fly-species-specific host plant databases of select quarantine-significant fruit fly pests of horticultural commodities; and, two, integrating host data from accessible taxonomic records, i.e., Fruit Fly (Diptera: Tephritidae) Databases developed through the USDA-ARS Systematic Entomology Laboratory. The former provides comprehensive host data for specific tephritid fruit fly species of economic importance, while the latter provides a broad background of hosts of fruit flies of the world. For the comprehensive databases, we acquired pertinent literature worldwide through the use of searchable databases, e.g., Agricola, CAB Abstracts, and Scopus, accessible through DigiTop, USDA's digital desktop library. Additionally, pertinent data were obtained from searches of the USDA-APHIS pest interception databases. Retrieved publications or references were classified as providing field infestation data, laboratory infestation data, or as just listing a fruit or vegetable as a host without providing any supporting data. For field infestation data, and, for some species, laboratory infestation data as well, a succinct summary was prepared.

**Results:** The Compendium of Fruit Fly Host Information (CoFFHI), an online searchable host plant database, was first released online in August 2015 (Edition 1.0), with subsequent release of an expanded version in September, 2016 (Edition 2.0) (available at <https://coffhi.cphst.org/>). It provides host information for the following fruit fly species of economic importance: the Mexican fruit fly, *Anastrepha ludens* Loew; the carambola fruit fly, *Bactrocera carambolae* Drew & Hancock; the guava fruit fly, *Bactrocera correcta* (Bezzi); the melon fly, *Bactrocera cucurbitae* (Coquillett); the oriental fruit fly, *Bactrocera dorsalis* (Hendel); *Bactrocera latifrons* (Hendel); *Bactrocera pedestris* (Bezzi); the *Bactrocera tau*

complex; the peach fruit fly, *Bactrocera zonata* Saunders; the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann); and the apple maggot fly, *Rhagoletis pomonella* (Walsh).

**Conclusions:** CoFFHI, Edition 2.0, is a primary reference on host plants of tephritid fruit fly species of economic importance. It is designed to enable regulatory scientists and regulatory officials to assess the risk of fruit flies in fresh horticultural commodities; to support regulatory decision-making during fruit fly emergency action and quarantine programs; to serve as a decision tool in the design and implementation of effective fruit fly detection, monitoring, suppression, and eradication programs of USDA and various state regulatory agencies; and, to assist in the development of protocols that prevent the introduction and establishment of exotic fruit flies that pose significant threats to U.S. agriculture and natural resources. Enhancing both the breadth and depth of summarization of the host plants of tephritid fruit flies in CoFFHI is an ongoing process. Subsequent releases of CoFFHI are expected to both augment and update data coverage for tephritid fruit fly species already covered in CoFFHI, Edition 2.0, as well as summarize host plant data for other fruit fly species, with a focus on *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus* and *Rhagoletis* species of economic importance.

**Keywords:** *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus*, host status, online searchable database, *Rhagoletis*.

## Introduction

Tephritid fruit flies are serious agricultural pests because of their direct damage to fruit through oviposition and larval feeding which can lead to both loss of product for local consumption as well as implementation of regulatory restrictions on the movement of commodities across national and international borders. Overall, tephritid fruit flies impose enormous constraints on the diversification of agricultural production and expansion of agricultural trade around the world. Knowledge of the host status of different fruits and vegetables is needed in assessing the risk of these fruit fly species in imported and exported fruit and vegetable commodities and for developing systems approaches and other mitigation measures to facilitate global trade. Rigorous quarantine procedures are currently enforced to prevent domestic and transnational spread of economically significant fruit flies. Accessible and reliable information facilitating the determination of the regulatory host or non-host status of the “recognized” host plants of fruit flies is essential to the successful implementation of risk mitigation protocols.

There has been a long history of developing lists of host plants of tephritid fruit flies of quarantine significance. These lists have needed to be updated over time as further documentation of host status has accumulated. Liquido et al. (1991, 1998) published results of a worldwide search for documentation of host plants of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). This host plant database, developed both in hard copy and electronic forms, has been a primary reference for regulatory officials for assessment of risk and for the development of mitigation protocols for Mediterranean fruit fly in interstate and international

movements of fruit and vegetable commodities. This database was released as an online interactive application in March, 2013 (MEDHOST, Version 1.1: <http://medhost.cphst.org/>) (Liquido et al., 2013). An updated version of MEDHOST (2.0) was released on June 23, 2014 (Liquido et al., 2014). MEDHOST 2.0 incorporated data from publications published after the initial Mediterranean fruit fly host summarization was completed (1997) as well as interception data which had not been included in the initial database. The updated version also included host plant images for most of the included plant hosts, PDFs of most of the source documents (accessible to USDA employees, but not to all users) and the ability to search the host plant database by host plant synonyms, authors, countries with field infestation, or host plant family. A further update, MEDHOST Version 3.3, was released in November 2016 (Liquido et al., 2016).

In addition to Mediterranean fruit fly, there are a number of other tephritid fruit fly species of quarantine significance which pose threats to the health of U.S. agriculture and natural resources, including a number of highly invasive species belonging to the genera *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus*, and *Rhagoletis*. USDA has a “strategic goal to develop support tools to reduce the introduction and prevent the establishment of exotic pests,” including quarantine-significant fruit flies. Building on the experience of developing a comprehensive online accessible, searchable database for hosts of Mediterranean fruit fly, the database was expanded to incorporate up-to date host plant data for multiple tephritid fruit fly species of quarantine significance, leading to the development of a comprehensive online accessible, searchable database for hosts of tephritid fruit flies of quarantine significance, the USDA Compendium of Fruit Fly Host Information, or in short CoFFHI <<https://coffhi.cphst.org/>> (USDA, 2016). Listed below are methods that were used in developing this compendium, followed by a summary of the contents of CoFFHI, Edition 2.0.

## Materials and Methods

### *Strategies used for summarizing host plant data*

We pursued the task of developing a host information system by utilizing two complementary strategies: one, developing comprehensive, online accessible fruit-fly-species-specific host plant databases of select quarantine-significant fruit fly pests of horticultural commodities; and, two, integrating host data from accessible taxonomic records, i.e., Fruit Fly (Diptera: Tephritidae) Databases ([www.sel.barc.usda.gov/diptera/tephriti/tephriti.htm](http://www.sel.barc.usda.gov/diptera/tephriti/tephriti.htm)).

### *Selection of fruit fly species for which comprehensive host plant data is included in CoFFHI*

The initial selection of tephritid fruit fly species for which host summarization would be focused was based on deliberations in November and December 2010 among USDA-APHIS-Plant Protection and Quarantine (PPQ) staff in Raleigh NC and Riverdale MD. The initial priority species were: the Mexican fruit fly, *Anastrepha ludens* (Loew); *Anastrepha* spp.; the melon fly, *Bactrocera cucurbitae* (Coquillett); the oriental fruit fly complex, *Bactrocera dorsalis* (Hendel) complex; the peach fruit fly, *Bactrocera zonata* (Saunders); and the

Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Subsequent needs in developing risk assessment and mitigation of fruit flies in various commodities have led to the prioritization of additional fruit fly species.

### *The Tephritidae Databases*

The Fruit Fly (Diptera: Tephritidae) Databases compile fruit fly taxonomic and host plant information in databases developed by Allen Norrbom (USDA-ARS, Systematic Entomology Laboratory [SEL]) and colleagues. They were previously available on the SEL website, but have been incorporated into CoFFHI so that they are available on a more reliable server and are more visible in conjunction with the other CoFFHI databases. The taxonomic database, originally developed as part of the Biosystematic Database of World Diptera (currently Systema Dipterorum, <http://www.diptera.org/>), now includes over 8,000 valid and invalid scientific names for nearly 5,000 currently recognized fruit fly species. Its host plant database comprises over 30,000 records. While host plant records by species are not comprehensive, the Tephritidae Databases provide taxonomic and geographic distribution information for all fruit fly species and document most if not all of the known fruit fly/host plant relationships. It is particularly useful to document the poorly known or minor pests which could gain major pest status, plausibly from host and geographic range expansion as a probable consequence of adaptive “host switching” due to limited host resources, inter-species competition, or climate change. It should also be noted, however, that the Tephritidae Databases, and particularly the host database, are working tools that are in a continuous state of development, thus not all records have been fully verified.

### *Standardization of Host Plant Taxonomy*

The taxonomy of the recorded host plants was verified according to current botanical classification using the USDA-ARS Germplasm Repository Information Network (GRIN, <http://www.ars-grin.gov/>); BONAP's Taxonomic Data Center (<http://bonap.net/tdc>); Tropicos (<http://www.tropicos.org/>), The Plant List (<http://www.theplantlist.org/>); and, the Global Biodiversity Information Facility (<http://www.gbif.org/>). The taxonomic information includes valid botanical names (genus, species, and author[s]; synonym[s]) and common name[s] in different languages. Host plant distribution is categorized either as native, introduced, cultivated, or present.

### *Acquisition of Host Plant Data*

The first step involved identification and acquisition of pertinent literature worldwide that provided data on the status of fruits and vegetables as hosts. Such references were acquired from publications indexed in searchable databases, e.g., Agricola, CAB Abstracts, Entomology Abstracts, Zoological Record, and Scopus, accessible through DigiTop, USDA's digital desktop library. Host listings of various state, national and international regulatory agencies were also obtained. Full length manuscripts not available directly through Digitop were requested from the National Agricultural Library. Additionally, pertinent data were



obtained from searches of the USDA-APHIS pest interception databases and comparable databases from other countries, when available.

### *Classification of Host Plant Data*

Retrieved publications or references were classified as providing field infestation data, laboratory infestation data, or as merely listing a fruit or vegetable as a host without providing any supporting data (“listing only”). For field infestation data and, for some species, laboratory infestation data as well, succinct summaries of the levels of infestation were prepared. These summaries include, where available, such details as the total number of samples collected, the total number of fruit collected, the total weight of fruit collected, the number of fruit flies recovered per kg of fruit and per kg of infested fruit, and the number of fruit flies recovered per infested fruit or per fruit.

## **Results and Discussion**

CoFFHI, Edition 1.0, was released in August 2015. In addition to including access to the Tephritidae Databases, it also provided comprehensive documentation of host records for *C. capitata*, *B. latifrons*, *B. carambolae*, and *B. correcta*. Also included were updated host lists for oriental fruit fly and melon fly. Edition 1.0 was since expanded in the September 2016 release of Edition 2.0 (available online at <https://coffhi.cphst.org/>, once permission for access is requested and received). Edition 2.0 has succinct infestation summaries for both laboratory and field infestations for *B. correcta* and *B. cucurbitae*, as well as succinct infestation summaries for field infestations in select plant families by *B. dorsalis*. Also added in are literature citations for references that provide field infestation data for *A. ludens*, *B. carambolae*, *B. pedestris*, the *B. tau* complex, and *B. zonata*. Additionally, provisional lists of suitable hosts and undetermined hosts are provided for all included fruit fly species as well as a provisional suitable host list for *R. pomonella*. Plant species are considered to be “suitable” hosts if there are confirmed infestation records under natural field conditions. Plant species are considered to be “undetermined” hosts if there is no validated record of infestation under natural field conditions, and its association with the fruit fly species is based on recorded laboratory infestation, interception at a port of entry, or a mere listing as a host without any accompanying verifiable data. Table 1 lists the numbers of suitable hosts and undetermined hosts for each of the fruit fly species included in CoFFHI, Edition 2.0, as well as the number of new host plant species that were identified through the host summarization efforts associated with developing CoFFHI.


**Table 1.** Comparison of the host plant records of quarantine significant fruit flies (Diptera: Tephritidae) among the *USDA Compendium of Fruit Fly Host Information* (CoFFHI, Edition 2.0, <https://coffhi.cphst.org/>), previous USDA official fruit fly host lists and the 7 CFR *Federal Domestic Quarantine Subpart—Fruit Flies* §301.32-2 (a) *Regulated articles* (Data summarized in August, 2016).

Fruit Species <sup>1</sup>	Fly Source of Record Data	Host Suitable Hosts	Undetermined Hosts	Total Hosts	Number of Newly Identified Hosts, or Number of Documented Hosts <sup>2</sup>
<i>Anastrepha ludens</i>	CoFFHI	45	51	96	96
	eCFR (07/28/2016)	-	-	26	-
<i>Bactrocera carambolae</i>	CoFFHI	101	40	141	141
<i>Bactrocera correcta</i>	CoFFHI	73	0	73	73
<i>Bactrocera cucurbitae</i>	CoFFHI	136	137	273	166
	USDA, 1986	-	-	107	-
	eCFR (07/28/2016)	-	-	46	-
<i>Bactrocera dorsalis</i>	CoFFHI	478	149	627	363
	USDA 1958, 1978, 1979, 1983 and 1989b	-	-	264	-
	eCFR (07/28/2016)	-	-	92	-
<i>Bactrocera latifrons</i>	CoFFHI	59	20	79	40
<i>Bactrocera pedestris</i>	CoFFHI	26	2	28	28
<i>Bactrocera tau</i> complex	CoFFHI	77	30	107	107
<i>Bactrocera zonata</i>	CoFFHI	55	81	136	136
	eCFR (07/28/2016)	-	-	94	-
<i>Ceratitis capitata</i>	MEDHOST/CoFFHI	361	142	503	427
	I				
	eCFR (07/28/2016)	-	-	53	-
	USDA, 1982	-	-	76	-

<sup>1</sup> Highlighted species have no previously published USDA host lists.

<sup>2</sup> Refers to the number of reported host plants in CoFFHI minus the number of host plants stated in previous USDA host lists (for *B. cucurbitae*, *B. dorsalis*, and *C. capitata*), or total number of recorded host plants of fruit flies for which USDA has no published host lists (i.e. *A. ludens*, *B. carambolae*, *B. correcta*, *B. latifrons*, *B. pedestris*, *B. tau* complex and *B. zonata*).

Fig.1 shows a sample species-specific host plant report in CoFFHI; it has details of botanical information and data on infestation of *Citrus sinensis* (L.) Osbeck by Mediterranean fruit fly. Because of space limitation, the data in the *C. sinensis* sample host report is heavily truncated.



**USDA Compendium of Fruit Fly Host Information**

Host Plant Reports: Scientific Names: *Bactrocera zonata*  
Common Names: *Ceratitidis capitata*

Search by the first character of the: *C*  
Tephritidae Databases: *W X Y Z*

Select a Scientific Host Name starting with C:  
*Citrus sinensis* (L.) Osbeck


**Host Information**

**Host** *Citrus sinensis* (L.) Osbeck

**Taxonomic #** 10782

**Taxonomic Source** GRIN

**Taxonomic Link** <https://npgsweb.ars-grin.gov/gringlobal/taxonomydetail.aspx?10782>

**Bugwood Image(s)** 

**Taxonomy** Plantae Magnoliophyta Magnoliopsida Sapindales Rutaceae

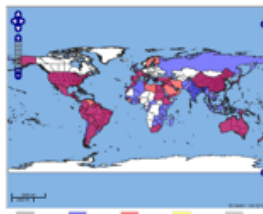
**Common Names** Apfelsine (German); Apfelsinenbaum (German); arancio dolce (Italian);

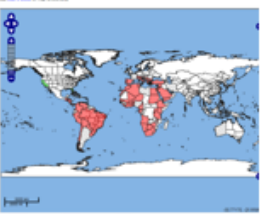
**Native** China (CABI); India (hort.purdue);

**Cultivated** Afghanistan (CABI); Albania (CABI); Algeria (CABI); Argentina (CABI);

**Present** Argentina (EOL); Australia (EOL); Azerbaijan (EOL); Azores (PGRForum);

**Countries with Field Infestation** Algeria; Argentina; Egypt; Greece; Hawaii; Israel; Italy; Libya; Morocco; Peru; Portugal; Seychelles; Spain; Tanzania; Tunisia; Uruguay

**Host Distribution Map** 

**Medfly Distribution Map** 

**Field Infestation** Liquido, N. J., R. T. Cunningham and S. Nakagawa. 1990: Hawaii, 35 of 479 collections with a total of 5,440 fruits were infested and produced 2.19 adults/kg of infested fruit.

**Laboratory Infestation** Papachristos, D. P., N. T. Papadopoulos, and G. D. Nanos. 2008: In separate, replicated experiments, punctured (2x with 1 mm diameter entomological pin) *C. sinensis* 'Xino Artas' fruits (20 - 28 fruits for each of three infestation regions) were artificially infested in either the flavedo, albedo, or pulp, by introducing 10 newly hatched larvae into each of the two holes. After larval introduction, each fruit was placed on a layer of soft paper in a muslin screened plastic cup. Pupae were collected daily and held individually in Petri dishes at  $24 \pm 1^\circ\text{C}$  ( $70 \pm 5\%$  RH; photoperiod of 16:8 [L:D] h) until adult emergence. *Ceratitidis capitata* survival through pupation, when reared on *C. sinensis* 'Xino Artas' fruits, averaged  $8.2 \pm 2.5\%$  and  $63.3 \pm 3.9\%$  for the albedo and pulp region, respectively.

**Interception Data** USDA 1939: *Ceratitidis capitata* was recovered from *C. sinensis* which originated from Brazil and intercepted at a port in Louisiana (1 immature, in "quarters"; 1 immature, in ship's stores); from Italy and intercepted at a port in New York (1 immature, in mail; 1 immature, in baggage; 1 immature, in ship's stores); and from Spain and intercepted at a port in Louisiana (1 immature, in ship's stores), New York (1 immature, in ship's stores), and Puerto Rico (1 immature, in ship's stores) between July 1, 1936 to June 30, 1937. Taxonomic identification was done by agricultural specialists of the states of California, Florida, and Hawaii, and the Bureau of Entomology and Plant Quarantine, USDA.

**Listing Only** \*Appiah, E. F., K. Afreh-Nuamah, and D. Obeng-Ofori. 2009; \*Cheikh, M., J. F. Howell,

**References** Papachristos, D. P., N. T. Papadopoulos, and G. D. Nanos. 2008. Survival and development of immature stages of the Mediterranean fruit fly (Diptera: Tephritidae) in citrus fruit. *Journal of Economic Entomology* 101: 866-872.

USDA. 1939. List of Intercepted Plant Pests, 1937. List of pests recorded during the period July 1, 1936 to June 30, 1937, inclusive, as intercepted in, on, or with plants and plant products entering United States territory. Service and Regulatory Announcements. United States Department of Agriculture, Bureau of Entomology and Plant Quarantine. Washington, D.C.

\*Indicates synonym was used by author      \*\* Indicates synonym and GRIN verified scientific name was used by author      + Indicates only common name was used by author

**Fig. 1.** Sample host report from CoFFHI: *Citrus sinensis* (L.) Osbeck. The host plants of *Ceratitidis capitata* were accessed by first selecting *Ceratitidis capitata* from the Fruit Fly Species pull down menu. Then data on *Citrus sinensis* was selected from the Scientific Host Names selection box. Host plant information and infestation data are truncated because of space limitation. Each functionality in CoFFHI is explained in the text.

The host plant report has the following functionalities: host scientific name; taxonomic number in GRIN; taxonomic link to GRIN taxonomy; host family; associated pests; host common names; synonyms and hybrids (if applicable); host images; host geographic distribution and map; countries with field infestations; succinct summaries of field, forced field and laboratory infestation data from published literature; port interception summaries; references that list the plant as a host, but provide no data; regulatory decisions and notes; and references. In addition to the species-specific host report page, there is a comprehensive host report output option to view information for all associated pests. The described functionalities are the same with the exception of countries with field infestations and succinct summaries sections. The countries are subdivided by pest. The succinct summaries for each infestation type are further categorized by pest. We suggest that interested readers log into CoFFHI Edition 2.0 to fully appreciate these functionalities, which are further described below.

### *Host Scientific Name*

The host's scientific name, taxonomic number, taxonomy, synonyms, hybrids and associated pests are stated at the beginning of the host report page. The host's scientific name is the name of the host report page. The taxonomic number is derived from USDA-ARS Germplasm Repository Information Network (GRIN, <http://www.ars-grin.gov/>). BONAP's Taxonomic Data Center (<http://bonap.net/tdc>), Tropicos (<http://www.tropicos.org/>), The Plant List (<http://www.theplantlist.org/>), and the Global Biodiversity Information Facility (<http://www.gbif.org/>) are used as taxonomic sources if the needed taxonomic information is not available in GRIN. A taxonomic link is provided specifying the source of the taxonomic number. Synonyms and cross parentage of each host plant's valid binomial names are specified, allowing users to search by synonym or hybrid. A search performed using a synonym or hybrid will redirect users to its host report page based on its currently valid scientific name. Each host plant's common names are primarily derived from GRIN and the cited publication. The language or country of origin of each common name derived from GRIN is provided (enclosed in parentheses), whenever available. The common names and synonyms are displayed in block format or compressed view. One can click on the "*Expanded view*" to see the common names in a list format with each common name or synonym on a separate line.

### *Host Images*

Host images are displayed according to their sources, which include USDA-NRCS PLANTS database (<http://plants.usda.gov/java/>), Bugwood (<http://images.bugwood.org/>), Tropicos (<http://www.tropicos.org/Home.aspx>), African Plants (<http://www.africanplants.senckenberg.de/>), Bishop Museum (<http://www.bishopmuseum.org/research/natsci/botany/botdbhome.html>), Forest and Kim Starr (<http://www.starrenvironmental.com/resources/>), and other properly acknowledged sources. Clicking on "*Play slideshow*" will cycle through the images within the enlarged picture section. There is an option to download the image that is currently displayed in the

enlarged picture section. Downloading the image displays the actual image size and lists the image's source and credits.

### *Host Distribution*

The host distribution categories are native, cultivated, present, and introduced. Because these categories are those used by the cited sources, the present, introduced and cultivated categories could have been used interchangeably without distinction. In many cases, the combination of “present” and “cultivated” provides a clear worldwide host distribution. Each country in the distribution list has a hyperlink to the source of the information.

### *Countries with Field Infestation*

The *Countries with a Field Infestation* section lists countries known to have fruit fly field infestations that were documented within the field infestation summaries. This section categorizes countries by pest when viewing the comprehensive host report page. The countries are displayed in block format called compressed view, but one can click on “*Expanded view*” to see the countries in a list format with each country on a separate line.

### *Host Distribution Map*

The host distribution map is a layered map. Different colors are used to show the countries where the host fruit is native, cultivated, present, or introduced, or any combination of these distribution categories. On the upper left side of the map are the zoom function and the directional arrows to move the map. On the upper right side of the map is a blue, “clickable” plus sign. Clicking on the plus sign displays the map overlays of host plant distribution, i.e., native, cultivated, present and introduced. Users can select or deselect the informational layers on the map. On the lower right side of the map is a blue, “clickable” plus sign. Clicking on the plus sign, while the map is zoomed in, brings up a small world map. A dotted red box within the small map shows the area in the world that the host distribution map is currently zoomed into. One can move to another location in the world while zoomed in by clicking on a new area on the mini world map. Using the mini world map feature to see another area on the map saves time by skipping the steps of zooming out to see another area of the world, re-centering the map, and then zooming in again. When the user would like to close the map overlays or mini world map panels, the user can click on the white minus sign located on the upper right or lower right side of the panel, respectively.

### *Field and Laboratory Infestation Data*

The field and laboratory infestation data are succinct summaries of the level of fruit fly infestation in a host fruit obtained either from field surveys or from laboratory experiments. Each succinct summary references the scientific sources or authors of the summarized information.

### *Interception Data*

The interception data are derived from USDA “List of Intercepted Plant Pests (LIPP)” and USDA PestID (<https://aqa.aphis.usda.gov/aqa/>), and comparable databases from other countries, when available.

### *Listing Only*

A listing only reference informs users that a scientific source or an author records a fruit or vegetable as a fruit fly host without providing any accompanying infestation data.

### *Regulatory Decisions and Notes*

This portion of the database integrates regulatory decisions made by USDA on specific host fruit commodities, which may include: “removing” or “including” a fruit fly as a quarantine pest following the commodity pathway; modifying quarantine protocol or a work plan based on conditional non-host status, thereby allowing movement of a previously regulated commodity; or requiring quarantine treatment to a previously considered non-host commodity that is currently being regulated as a suitable host requiring mitigation.

### *References*

Each host report page provides a complete bibliographic list of data sources. Scientific sources or publication articles are archived as PDF files. Each reference has a “clickable” PDF icon which can be used to open the PDF file, though the activation of this feature is dependent on the user’s level of access.

### *Search*

Users can search the fruit fly species specific database by author and infestation type using the main *Search* tab for this database that appears on the navigation bar. Alphabetical searches for comprehensive host report pages, either through the host plant’s scientific or common names, are performed by browsing the corresponding dropdown menu under the *Host Plant Species* tab located on the top navigation bar. Users can also search through all the scientific and common names by clicking on the *Host Plant Species* tab located on the top navigation bar and selecting either the scientific or common names radio button. Similar radio button searches by host plant’s scientific and common names are available for fruit fly specific host report pages after selecting a specific fruit fly database from the dropdown menu under the *Fruit Fly Species* tab located on the top navigation bar. Different search options are described more fully below.

#### *Search by Scientific Names*

Click on *Scientific Names* from the *Host Plant Species* tab dropdown menu and A-Z letters appear horizontally. Searching and obtaining a comprehensive host plant report can be achieved by clicking on the first letter of a particular host plant’s genus name, which opens a dropdown menu listing of all scientific host plant names starting with that letter. By selecting

a scientific host plant name from the dropdown menu, a comprehensive host plant report is generated.

To browse all scientific names for a comprehensive report page, click on the *Host Plant Species* tab located on the top navigation bar. Under '*Host Plant Reports*,' click the *Scientific Names* radio button. A list of all host plant species in the database appears. Scroll through the list to select a host plant. To quicken the scrolling process, click in the box on the line '*Select a Host Name below*,' then type the first letter of the genus of the species. The selector will move down the list to a genus name starting with that letter. Type the first several letters of the genus quickly and the selector will move to the genus name beginning with those letters. Select a host plant to generate the host report page under the search area. Multiple host pages can be viewed by holding down the control key while selecting multiple host names.

To browse all scientific names for a fruit fly species specific report page, click on the fruit fly species of interest from the dropdown menu under the *Fruit Fly Species* tab located on the top navigation bar. The user is first redirected to the fruit fly's database title page displaying the title and authorship of the USDA provisional host list associated with the information found within the database. Clicking on *Continue* brings one to the selected fruit fly database. Under '*Select a Host Scientific Name or Host Common Name to view the Host Report for [specified fruit fly]*,' click the *Scientific Names* radio button. A list of all host plant species in the database appears. Selecting a host plant from the list functions in the same manner described previously by scrolling or typing.

#### *Search by Common Names*

Click on *Common Names* from the *Host Plant Species* tab dropdown menu and A-Z letters appear horizontally. Searching and obtaining a comprehensive host plant report can be achieved by clicking on the first letter of a particular host plant's common name, which opens a dropdown menu listing all common host plant names starting with that letter. The dropdown menu lists the common host plant names followed by the language of the common name and the common name's scientific name (both enclosed in parentheses).

To browse all common names for a comprehensive report page, click on the *Host Plant Species* tab located on the top navigation bar. Under '*Host Plant Reports*,' click the *Common Names* radio button. Selecting a host plant from the list functions in the same manner described previously by scrolling or typing.

To browse all common names for a fruit fly species specific report page, click on the fruit fly species of interest from the dropdown menu under the *Fruit Fly Species* tab located on the top navigation bar. Click on *Continue* at the bottom of the fruit fly species title page to continue into the fruit fly specific database. Under '*Select a Host Scientific Name or Host Common Name to view the Host Report for [specified fruit fly]*,' click the *Common Names* radio button. A list of all host plant species in the database appears. Selecting a host plant from the list functions in the same manner described previously by scrolling or typing.

*Main Search: CoFFHI.* Open the CoFFHI search page by clicking on the *CoFFHI* dropdown menu located under the *Search* tab on the top navigation bar. Here searches can be performed by author according to their port interception record, laboratory and/or field infestation data, or host listing record. Start typing the name of the author to generate a dropdown menu listing authors beginning with the typed letters. The user can then select an author from the dropdown list. Click *Search* after the desired search fields have been selected. The search results are displayed in a table format with the columns containing host scientific names, authors and infestation summaries. Furthermore, the total number of infestation records in the table, by infestation category, i.e., field, laboratory, interception and “listing only”, are displayed on the upper right side of the screen.

*Main Search: Tephritidae Databases.* Open the Tephritidae Databases search page by clicking on the *Tephritidae Databases* dropdown menu located under the *Search* tab on the top navigation bar. The user is first redirected to the title page of Allen Norrbom’s Tephritidae Databases. Clicking on *Continue* brings one to the Tephritidae Databases. Here searches can be performed by fruit fly species or host plants to view a full report or by host plant species to view a table of associated fruit fly species, countries of reported infestations, and citations.

#### *Tephritidae Databases Full Report Searches*

To view a report by host, click on the *Host* link within the ‘*Search by Hosts to view a full report*’ option. Selecting a host plant’s scientific name from the list functions in the same manner described previously by scrolling or typing. Once a host is selected, full host reports are generated per cited reference. Allen Norrbom’s full reports provide host information, verification summary, and references. Depending on the information available in the literature, ‘*Host Information*’ includes host scientific name, host common names, host family, pest scientific name, plant REF (taxonomic source), check date (date taxonomic source was used), GRIN nomen (taxonomic number), native distribution, host origin, and country of infestation. ‘*Norrbom’s Verification*’ summary may include the following data: citation, citation source, pest name, host name, plant part infested, record basis, infestation country, and whether the source was verified by Allen Norrbom. The literature source that provided the information found under the host information and verification summary sections are cited under the ‘*References*’ section. Similarly, to view a report by pest, click on the *Fruit Fly Species* link within the ‘*Search by Fruit Fly Species to view a full report*’ option. Select a pest from the drop down menu. The pest names are followed by the literature source within parentheses. Once a pest is selected, full pest reports are generated per cited reference containing pest information, verification summaries and references.

#### *Tephritidae Databases Table Searches*

To view associated pests, citations and/or countries of reported infestations by host, click on the *Host* link within either the ‘*Search by Hosts to view a list of associated fruit fly species and citations*’ or ‘*Search by Hosts to view a list of fruit fly species, countries and citations*’ option. Select a host from the dropdown menu. The search results are displayed in a table



format with the columns containing host scientific names, fruit fly, reference, and possibly country depending on the search option selected. Furthermore, to view the full report from one of those table results, click on the corresponding host link under the '*Host Scientific Name*' column.

### *Provisional Host Lists*

To view USDA provisional host lists created to provide data for CoFFHI, click on the *Provisional Host Lists* dropdown under the *Fruit Fly Species* tab located on the top navigational bar.

### *Printing CoFFHI Host Report Pages*

There are three options to print the host plant report: printer friendly, Word compressed and Word expanded. If the user chooses to include host plant images and distribution maps in the print output, the user should click on printer friendly version located on the upper right section of the page. After the printer friendly version is displayed, right click on the screen and select print. If, however, the user opts not to include host plant pictures and distribution maps in the print, the user should click on *Save as Word compressed* or *Save as Word expanded* located on the upper right section of the page. The *Save as Word compressed* and *Save as Word expanded* differ on the display format of the host plant's common names on the host report page. The compressed view saves space by listing the common names in a block format. The expanded view lists each common name on a new line in a list format. The user is given a choice to open or save the Word document. The Word document is saved as the host's scientific name followed by Report or Expanded Report in the user's computer download folder. If the Word document does not automatically open, click on the computer *Start Menu* and then click the *Downloads* tab. Double click on the Word document file titled after the host's scientific name. Select *Print* under the *File* tab within the Word document.

## **Conclusions**

CoFFHI, Edition 2.0, is a USDA information technology asset, one of the web applications comprising the *Export Pest Information and Prediction System* (EPIPS). As a primary reference on host plants of tephritid fruit fly species of economic importance, CoFFHI, Edition 2.0, is designed to enable regulatory scientists and regulatory officials to assess the risk of fruit flies in fresh horticultural commodities, to support regulatory decision-making during fruit fly emergency action and quarantine programs, to serve as a decision tool in the design and implementation of effective fruit fly detection, monitoring, suppression, and eradication programs of USDA and various state regulatory agencies and to assist in the development of protocols that prevent the introduction and establishment of exotic fruit flies that pose significant threats to U.S. agriculture and natural resources, especially in southern-situated states of the U.S., e.g., California, Texas, Florida, that are vulnerable to invasion by alien fruit flies. CoFFHI is a vital tool in achieving the core mission of USDA-APHIS-PPQ in preventing the introduction and establishment of exotic fruit flies.

Enhancing both the breadth and depth of summarization of the host plants of tephritid fruit flies in CoFFHI is an ongoing process. Subsequent releases of CoFFHI are expected to both augment and update data coverage for tephritid fruit fly species already covered in CoFFHI, Edition 2.0, as well as summarize host plant data for additional fruit fly species, with a focus on *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus* and *Rhagoletis* species of economic importance.

## Acknowledgments

CoFFHI Edition 2.0 is made possible by the technical assistance of present and previous NCSU-CIPM, USDA-APHIS-CPHST and USDA-ARS Research Assistants and University of Hawaii (UH)- Hilo students: Megan A. Hanlin, Amanda L. Birnbaum, Kelly A. A. Nakamichi, Jess R. Inskeep, Alexander J. Ching, Ashley McGuigan, Kyle Kumashiro, Noah J. Hegerfeldt, April M. Greenwell, Kristine G. Ayson, and John E. Montoya (CIPM); Sarah A. Marnell and Rick S. Kurashima (CPHST); Charmaine D. Sylva (ARS); and Sierra V. Salazar, Monique R. Walls, and Leslie L. Wynne (UH-Hilo). Sandra Sferrazza (CIPM) provided the programming support. To all of them, *Mahalo!*

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ISBN 978-616-358-207-2



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