Universitat de Lleida

BIOLOGY STUDIES AND IMPROVEMENT OF *Ceratitis capitata* (Wiedemann) MASS TRAPPING CONTROL TECHNIQUE

Inmaculada Esther Peñarrubia María

BIOLOGY STUDIES AND IMPROVEMENT OF *Ceratitis capitata* (Wiedemann) MASS TRAPPING CONTROL TECHNIQUE

Ph.D.

Inmaculada Esther Peñarrubia María

Lleida, September 2010

UNIVERSITY OF LLEIDA

School of agricultural and forestry engineering of Lleida

Ph.D.

BIOLOGY STUDIES AND IMPROVEMENT OF *Ceratitis capitata* (Wiedemann) MASS TRAPPING CONTROL TECHNIQUE

Inmaculada Esther Peñarrubia María

Director: **Dr. L. Adriana Escudero Colomar**

Co-director: **Dr. Jesús Avilla Hernández**

Lleida, September 2010

TRIBUNAL MEMBERS

Chairwoman

Dr. María José Sarasúa
Universitat de Lleida

Secretary

Dr. Manel Ribes
Universitat de Lleida

Members

Dr. Fabio Molinari
Università Cattolica S.C. – Facoltà di Agraria

Dr. Giovanni Burgio
Università di Bologna

Dr. Francisco Beitia
Instituto Valenciano de Investigaciones Agrarias (IVIA)

Supply members

Dr. Matilde Eizaguirre
Universitat de Lleida

Dr. Maria Teresa Martínez
Institut de Recerca i Tecnologia Agroalimentària (IRTA)

ACKNOWLEDGEMENTS

The present work has been supported by the INIA Research Project RTA2006-00092-00-00, the CDTI project TQS-20060327 and several institutions: IRTA-Experimental Station Mas Badia, UDL-IRTA and CIRAD-La Réunion.

This report would not have been possible without the professional support of:

- Dr. L. Adriana Escudero (IRTA) and Dr. Jesus Avilla (UdL-IRTA), who expressed confidence in my work and offered much help and advice over the last four years.
- Dr. Nikos Papadopoulos (University of Thessaly) and Dr. Serge Quilici (CIRAD-La Réunion), who accepted me as a member of their research teams.
- Mr. Trevor Rushby, thanks for his help with English grammar and his friendship.

I would also like to thank:

- All the team from the Research centre IRTA-Experimental Station Mas Badia, for their sympathy in my everyday work, especially to Josep Maria Pagès and Marià Vilajeliu.
- Dr. María José Sarasúa, Dolors Bosch and Lluís Torres (UdL-IRTA), Dr. Silvia Abril and Natàlia Adell (UdG), Dr. Francisco Beitia (IVIA) and Lluís Batllori (Government of Catalonia) for their help and reliable advice.
- The team from SEDQ S.L. company, the fruit growers involved in our studies for allowing us to conduct trials in their orchards and the technicians Anna Cerdà, César Saiz, Gemma Esteva, María Carbó, Núria Madeo, Raúl Sanchez, Francesc Raset and Olivier Fages for making my job easier in the commercial orchards.
- Laura Boixó, Gerard Morales, Ferran Garcia-Molí, Albert Garriga, Marina Poch, Cesca Alcalà, Stella Papanastasiou, Chloé Schmitt and Jim Payet for their kind help with laboratory and field work throughout my time in the E. S. Mas Badia, in the University of Thessaly and in CIRAD-La Réunion.

I would like also to offer my special thanks to:

- My parents Adolfo Peñarrubia and Lali María, my sister Maite Peñarrubia, Toni Serrano and the Machordom's family for their enormous support and patience, not only during the thesis period.
- Jordi Machordom for his endless patience and his emotional support in our everyday life.
- My beloved four little nieces for being always so funny and charming.
- My friends Elisa Gibert, Jeny Muñoz, Edurne de Juan, Natalia Ureña, Mónica Martínez and Nati Iglesias for all the laughs we have had together.

I would like to dedicate this thesis to the memory of my grandfather,

Eliseo Peñarrubia, who taught me the pleasure of reading and writing.

ABSTRACTS

ABSTRACT

Ceratitis capitata (Wiedemann) (medfly), is considered to be one of the world's most destructive fruit pests because of its high capability to damage the production, its global distribution and its wide range of hosts. The development of an effective integrated pest management (IPM) model has been accepted as a plant protection strategy for sustainable farming in Europe. The objective of the present work was to study characteristics of the biology of the pest and to improve the mass trapping technique, included in IPM for the control of *Ceratitis* spp., in two different areas: Girona and La Réunion Island.

There is a lack of overwintering studies of medfly in the North-East extreme of Spain and as it is important to find out the conditions of overwintering of the different stages of development, several trials were carried out. All trials had a control under chamber conditions. Stages of larvae and pupae of medfly collected from infested apples survived the natural conditions of late autumn and early winter in the Girona fruit growing area but not through the entire winter period. Larval and pupa stages maintained in winter conditions developed more slowly when compared with individuals reared in controlled ones. Adults continued to emerge until mid January, so it was not possible to prove that adults found in the following spring-summer came from fruits infested in winter. No adult medfly emerged from pupae which spent all winter under natural conditions, due to several factors at subsoil level, including high number of cold hours below the threshold for pupae development. Medfly adults were unable to survive the entire winter season in the Girona area. Climatic conditions including daily temperature and high level of rainfall appeared to be involved in the mortality of adults during winter.

Studies based on several field trials of equipment used for the mass trapping of medfly conducted in Girona, were performed in the Island of La Réunion, where two *Ceratitis* spp. coexist. It was evaluated the effectiveness of trapping equipment used for *Ceratitis rosa* Karsch and *C. capitata* through comparative studies of trap types, attractants, insecticides and commercial complete equipments (systems). In addition to the two target species, other fruit fly species registered were: *Bactrocera cucurbitae* (Coquillett), *Neoceratitis cyanescens* (Bezzi), *Dacus ciliatus* Loew, *Bactrocera zonata* (Saunders), *Ceratitis catoirii* Guérin-Ménville and *Dacus demmerezi* (Bezzi), exposed in order of importance. The most captured species in all trials were *C. rosa* followed by *B. cucurbitae* and by *C. capitata*. The most effective traps for the capture of *C. rosa* and *C. capitata* were Maxitrap® and Tephri-trap®, which also captured higher number of *B. cucurbitae*. The most effective baits for the attraction of *C. rosa* were BioLure® Med Fly and BioLure® Unipak. Ferag® CC D TM, BioLure® Med Fly and BioLure® Unipak obtained low levels of medfly captures. The formulation tested of the insecticide deltamethrin 15 mg would be

a suitable substitute for DDVP, use of which has recently been banned in the EU. The systems including BioLure® Unipak+Tephri-trap®+DDVP and Ferag® CC D TM+Maxitrap®+DDVP performed effectively for *C. rosa* and *C. capitata*. These two systems and Cera trap® obtained the same results for *B. cucurbitae*. These results must be corroborated at a later date, using the available insecticides.

The aims of the next study were to find an insecticide and formulation for use in mass trapping, which is at least as effective as DDVP; to identify the optimal location of the insecticide in the trap and to ascertain the efficacy of the prototype carrying the selected insecticide used for mass trapping. Deltamethrin at 20 mg dose, impregnated by movement of the lid, is a possible substitute for DDVP, although in the previous trial, insecticide impregnated by movement in the base of the trap was found to have a slightly superior effectiveness. It is, therefore important that further studies be made of the optimum position of the insecticide in the traps. The plastic prototype with a formulation of deltamethrin 12 mg, demonstrated a highly efficient killing action at both low and high population levels in mass trapping against medfly.

The medfly has a major economic impact on the peach crop in the Mediterranean basin but very few studies of mass trapping have been conducted on them. The objective of the present work, therefore, was to optimize the application of the methodology on this particular crop. Several trials found that field colonization by the species usually started on the edge of the plots and from there, spread throughout the orchard. Host plants, species that provided refuge for birds and water courses located at borders of the plot, all appeared to be important factors in the spatial distribution of the pest and must always be considered when mass trapping is used. This technique is effective in the North-East of Spain in the control of medfly in peach orchards when the population level is low but when it is high it must be reinforced by chemical spraying. The proportion of traps to be checked was inversely related to the population density captured. When plots were larger than 1 ha, it would be enough to check 60% of the traps during the last 5 weeks of the ripening period, or 70% over the full period to ensure a reliable estimate of the population found.

RESUM

Ceratitis capitata (Wiedemann) (mosca de la fruita), està considerada a nivell mundial com una de les plagues més destructives de fruits degut a la seva elevada capacitat de causar danys en la producció, la seva distribució global i al seu ampli rang d'hostes. S'ha desenvolupat un model eficaç de control integrat de plagues (IPM), que ha estat acceptat a Europa com estratègia de protecció vegetal per a una agricultura sostenible. L'objectiu del present treball va ser l'estudi de característiques de la biologia de la plaga y la millora de la tècnica de captura massiva, inclosa en IPM per al control de *Ceratitis* spp., a dues àrees diferents: Girona y l'illa de La Reunió.

Degut a la mancança d'estudis d'hivernació de *C. capitata* a l'extrem nord-est d'Espanya i a la importància de conèixer les condicions en que passen l'hivern els diferents estadis de desenvolupament, es van dur a terme diversos assajos. Tots ells varen tenir el seu respectiu control sota condicions controlades. Els estadis de larva i pupa de mosca de la fruita obtinguts a partir de pomes amb símptomes d'atac van sobreviure condicions de finals de tardor i principis d'hivern de la zona fructícola de Girona, però no van sobreviure durant tot el període hivernal. Els estadis de larva i pupa mantinguts sota condicions hivernals es van desenvolupar més lentament que els individus mantinguts a condicions controlades. Els adults van emergir fins mitjans de gener, pel que no va ser possible provar que els adults trobats el període de primavera i estiu provinguessin de fruits atacats a l'hivern. Cap adult va emergir de les pupes disposades sota condicions naturals degut a diversos factors a nivell de subsòl, com l'elevat nombre d'hores de fred per sota del llindar de desenvolupament de pupa. A la zona de Girona, els adults de mosca de la fruita van ser incapaços de sobreviure durant tot l'hivern. Les condicions climàtiques incloent temperatura diària i alt nivell de pluviometria van afectar a la mortalitat dels adults durant l'hivern.

Es van dur a terme estudis a l'illa de La Reunió, on coexisteixen dues espècies de *Ceratitis*, basant-se en assajos de camp realitzats a Girona amb material de captura massiva contra *C. capitata*. Es va avaluar l'eficàcia del material de captura contra *Ceratitis rosa* Karsch y *C. capitata* mitjançant estudis comparatius de tipus de trampes, atraients, insecticides i equips comercials complerts (sistemes). A més de les dues espècies objectiu, es van comptabilitzar altres espècies de mosques de la fruita: *Bactrocera cucurbitae* (Coquillett), *Neoceratitis cyanescens* (Bezzi), *Dacus ciliatus* Loew, *Bactrocera zonata* (Saunders), *Ceratitis catoirii* Guérin-Ménville y *Dacus demmerezi* (Bezzi), citades per ordre d'importància. Les espècies més capturades en tots els assajos van ser *C. rosa* seguida per *B. cucurbitae* i *C. capitata*. Les trampes més eficaces per la captura de *C. capitata* i *C. rosa* van ser Maxitrap® i Tephri-trap®, essent també les que van capturar major número de *B. cucurbitae*. Els atraients més efectius per *C. rosa* van ser BioLure® Med Fly i BioLure® Unipak.

Ferag® CC D TM, BioLure® Med Fly i BioLure® Unipak van obtenir nivells baixos de captures de *C. capitata*. La formulació amb l'insecticida deltametrina 15 mg podria ser un substitut adequat pel DDVP, recentment prohibit a la UE. Els sistemes formats per BioLure® Unipak+Tephri-trap®+DDVP i Ferag® CC D TM+Maxitrap®+DDVP van ser efectius contra *C. rosa* i *C. capitata*. Aquests dos sistemes van obtenir els mateixos resultats per *B. cucurbitae*. En un futur aquests resultats haurien de ser corroborats emprant els insecticides disponibles per al seu ús.

Els objectius del següent estudi van ser trobar un insecticida tècnic i una formulació per al seu ús en captura massiva, que fossin al menys tan eficaços como el DDVP; identificar la posició òptima de l'insecticida dins la trampa i esbrinar l'eficàcia d'un prototip portador de l'insecticida seleccionat per ser emprat en captura massiva. La dosi de 20 mg de deltametrina impregnada per moviment de la tapa és un possible substitut del DDVP, tot i que a l'assaig previ l'insecticida impregnat per moviment a la base de la trampa va tenir una eficàcia lleugerament superior. Degut a això, serien necessaris més estudis sobre la posició òptima de l'insecticida dins la trampa. El prototip plàstic impregnat amb una formulació de deltametrina 12 mg va ser emprat en captura massiva contra *C. capitata* i va demostrar una acció insecticida altament eficaç tant a nivells poblacionals baixos com alts.

A la conca Mediterrània, *C. capitata* té un elevat impacte econòmic sobre el conreu del presseguer, sobre el que s'han realitzat molt pocs estudis de captura massiva. Per això, l'objectiu d'aquest estudi va ser optimitzar l'ús d'aquesta metodologia sobre presseguer. Diversos assajos van demostrar que generalment l'espècie iniciava la colonització de la parcel·la a una vora i d'allà s'estenia a la resta de superfície. Les plantes hoste, les espècies que proporcionen refugi per ocells i els cursos d'aigua localitzats a la perifèria de la parcel·la semblen ser factors importants en la distribució espaial de la plaga i haurien de ser considerats quan s'utilitzi la captura massiva. Aquesta tècnica és eficaç en presseguers del nord-est d'Espanya per al control de *C. capitata* a nivells poblacionals baixos, mentre que a nivells elevats és necessari reforçar-la amb tractaments químics. La proporció de trampes a revisar estava inversament relacionada amb la densitat poblacional capturada. Per tal d'estimar de manera fiable la població capturada, en parcel·les majors a una hectàrea seria suficient revisar el 60 % de las trampes durant les darreres cinc setmanes del període de maduració, o el 70 % durant tot el període.

RESUMEN

Ceratitis capitata (Wiedemann) (mosca de la fruta), está considerada a nivel mundial como una de las plagas más destructivas de frutos debido a su capacidad de dañar la producción, su distribución global y a su amplio rango de huéspedes. Se ha desarrollado un modelo eficaz de control integrado de plagas (IPM), que ha sido aceptado en Europa como una estrategia de protección vegetal para una agricultura sostenible. El objetivo del presente trabajo fue el estudio de características de la biología de la plaga y la mejora de la técnica de captura masiva, incluida en IPM para el control de *Ceratitis* spp., en dos áreas diferentes: Girona y la isla de La Reunión.

Debido a la falta de estudios referentes a cómo inverna *C. capitata* en el extremo noroeste de España y a la importancia de conocer las condiciones en que pasan el invierno los diferentes estadios de desarrollo, se realizaron varios ensayos. Todos ellos tuvieron su respectivo control bajo condiciones de cámara. Los estadios de larva y pupa de mosca de la fruta obtenidos a partir de manzanas con síntomas de ataque sobrevivieron condiciones de finales de otoño y principios de invierno en la zona frutícola de Girona, pero no sobrevivieron durante todo el período invernal. Los estadios de larva y pupa mantenidos bajo condiciones invernales se desarrollaron más lentamente que los individuos mantenidos en condiciones controladas. Los adultos emergieron hasta mediados de enero, por lo que no fue posible probar que los individuos encontrados en la siguiente primavera o verano provinieran de frutos atacados en invierno. De las pupas dispuestas bajo condiciones naturales no emergió ningún adulto, debido a diversos factores a nivel de subsuelo, como el elevado número de horas de frío bajo el umbral de desarrollo de pupa. En la zona de Girona los adultos de mosca de la fruta fueron incapaces de sobrevivir durante todo el invierno. Las condiciones climáticas, incluyendo temperatura diaria y un elevado nivel de pluviometría afectaron a la mortalidad de los adultos durante el invierno.

Se realizaron estudios en la isla de La Reunión, donde coexisten dos especies de *Ceratitis*, basándose en ensayos de campo llevados a cabo en Girona con material de captura masiva contra *C. capitata*. Se evaluó la eficacia del material de captura contra *Ceratitis rosa* Karsch y *C. capitata* mediante estudios comparativos de tipos de trampas, atrayentes, insecticidas y equipos comerciales completos (sistemas). Además de las dos especies objetivo, se contabilizaron otras especies de moscas de la fruta: *Bactrocera cucurbitae* (Coquillett), *Neoceratitis cyanescens* (Bezzi), *Dacus ciliatus* Loew, *Bactrocera zonata* (Saunders), *Ceratitis catoirii* Guérin-Ménville y *Dacus demmerezi* (Bezzi), citadas por orden de importancia. Las especies más capturadas en todos los ensayos fueron *C. rosa* seguida por *B. cucurbitae* y *C. capitata*. Las trampas más eficaces para la captura de *C. capitata* y *C. rosa* fueron Maxitrap® y Tephri-trap®, siendo también las que capturaron mayor número de *B.*

cucurbitae. Los atrayentes más efectivos para C. rosa fueron BioLure® Med Fly y BioLure® Unipak. Ferag® CC D TM, BioLure® Med Fly y BioLure® Unipak obtuvieron niveles bajos de capturas de *C. capitata*. La formulación con el insecticida deltametrina 15 mg podría ser un substituto adecuado para el DDVP, recientemente prohibido en la UE. Los sistemas formados por BioLure® Unipak+Tephri-trap®+DDVP y Ferag® CC D TM+Maxitrap®+DDVP fueron efectivos contra *C. rosa* y *C. capitata*. Estos dos sistemas obtuvieron los mismos resultados para *B. cucurbitae*. En un futuro estos resultados tendrían que ser corroborados utilizando los insecticidas disponibles para su uso.

Los objetivos del siguiente estudio fueron encontrar un insecticida técnico y una formulación para su uso en captura masiva, que fueran al menos tan eficaces como el DDVP; identificar la posición óptima del insecticida en la trampa y averiguar la eficacia de un prototipo portador del insecticida seleccionado para ser usado en captura masiva. La dosis de 20 mg de deltametrina impregnada por movimiento de la tapa es un posible substituto del DDVP, aunque en el ensayo anterior el insecticida impregnado por movimiento en la base de la trampa tuvo una eficacia ligeramente superior. Por lo tanto, serían necesarios más estudios sobre la posición óptima del insecticida dentro de la trampa. El prototipo plástico impregnado con una formulación de deltametrina 12 mg fue usado en captura masiva contra *C. capitata* y demostró una acción insecticida altamente eficaz tanto a niveles poblacionales bajos como altos.

En la cuenca Mediterránea, *C. capitata* tiene un elevado impacto económico sobre el cultivo del melocotón, sobre el cual se han realizado muy pocos estudios de captura masiva. Por ello, el objetivo de este estudio fue optimizar el uso de dicha metodología sobre melocotonero. Varios ensayos demostraron que generalmente la especie iniciaba la colonización de la parcela desde un borde y desde allí se extendía al resto de superficie. Las plantas huésped, las especies que proporcionan refugio para pájaros y los cursos de agua localizados en la periferia de la parcela parecen ser factores importantes en la distribución espacial de la plaga y deberían ser considerados cuando se emplee la captura masiva. Esta técnica es eficaz en melocotoneros del noreste de España para el control de *C. capitata* a niveles poblacionales bajos, mientras que a niveles elevados es necesario reforzarla con tratamientos químicos. La proporción de trampas a revisar estaba inversamente relacionada con la densidad poblacional capturada. Con el fin de estimar de manera fiable la población capturada, en parcelas mayores a una hectárea bastaría con revisar el 60 % de las trampas durante las últimas cinco semanas del período de maduración, o el 70 % durante todo el período.

GENERAL INDEX

GENERAL INDEX

ACKNOWLEDGEMENTS.. 1

ABSTRACTS.. 3

1. INTRODUCTION.. 15

2. GENERAL OBJECTIVES... 51

3. CHAPTER I: SURVIVAL OF WILD LARVAE AND OVERWINTER OF PUPA AND ADULT STAGES UNDER NATURAL WINTER CONDITIONS OF THE GIRONA AREA…………………………………………………………………. 55

4. CHAPTER II: EVALUATION OF MASS TRAPPING EQUIPMENT AGAINST *CERATITIS* SPP. ON THE ISLAND OF LA RÉUNION.................................. 93

5. CHAPTER III: INSECTICIDES FOR USE IN MASS TRAPPING CONTROL TECHNIQUE FOR *CERATITIS CAPITATA*…………………………………......... 127

6. CHAPTER IV: MASS TRAPPING CONTROL TECHNIQUE FOR *CERATITIS CAPITATA* IN PEACHES…………………………………………………….... 155

7. GENERAL DISCUSSION... 193

8. GENERAL CONCLUSIONS.. 205

1. INTRODUCTION

INDEX

1 INTEGRATED PEST MANAGEMENT 19

1.1 CONCEPT OF INTEGRATED PEST MANAGEMENT 19

1.2 IPM AND IFP IN SPAIN 20

1.3 PESTS IN FRUIT-TREES FROM CATALONIA 21

2 IMPORTANCE OF *CERATITIS CAPITATA* 21

3 TAXONOMY OF *CERATITIS CAPITATA* 22

4 BIOLOGY AND OTHER CHARACTERISTICS OF MEDFLY 23

5 DAMAGE AND ECONOMIC IMPORTANCE 24

6 CURRENT AND UNDER DEVELOPMENT CONTROL METHODS 25

6.1 CHEMICAL CONTROL 25

6.1.1 SPINOSAD 26

6.2 CONTROL WITH BOTANICAL INSECTICIDES 28

6.2.1 *CESTRUM PARQUI* 28

6.2.2 *CITRUS AURANTIUM* 28

6.2.3 *CITRUS LIMON* 28

6.2.4 *SOLANUM GILO* 28

6.2.5 *THYMUS CAPITATUS*, *THYMUS HERBA-BARONA* AND *CINNAMOMUM ZEYLANICUM* 28

6.3 BIOLOGICAL CONTROL 29

6.3.1 PARASITOIDS 29

6.3.2 PREDATORS 30

6.4 MICROBIAL CONTROL 30

6.4.1 *BACILLUS THURINGIENSIS* 30

6.4.2 *BEAUVERIA BRONGNIARTII* AND *B. BASSIANA* 31

6.4.3 *METARHIZIUM ANISOPLIAE* 31

6.4.4 *MUCOR HIEMALIS* 31

6.5 CONTROL WITH NEMATODES 31

6.5.1 *HETERORHABDITIS* SPP. 31

6.5.2 *STEINERNEMA* SPP 31

6.6 CONTROL WITH PHOTOINSECTICIDES 32

6.7 CONTROL THROUGH ATTRACT AND STERILIZE 32

6.7.1 LUFENURON 32

6.7.2 OTHER CHEMOSTERILANT AGENTS 33

6.8 CONTROL THROUGH ATTRACT AND KILL 33

6.9 CONTROL THROUGH STERILE INSECT TECHNIQUE 34

6.9.1 NORMAL SIT 34

6.9.2 SIT WITHOUT IRRADIATION 34

6.10 CONTROL THROUGH INCOMPATIBLE INSECT TECHNIQUE 34

6.11 CONTROL THROUGH FOOD BASED ATTRACTANTS AND MASS TRAPPING TECHNIQUE 35

6.12 AGRONOMICAL MEASURES 37

6.13 POST-HARVEST CONTROL 37

6.13.1 COLD TREATMENTS 37

6.13.2 WARM TREATMENTS 37

6.13.3 ARTIFICIAL VISION SYSTEM 38

6.13.4 IONIZING IRRADIATION 38

6.13.5 INSECTICIDAL ATMOSPHERE 38

6.14 COMBINATION OF SEVERAL CONTROL METHODS 38

7 REFERENCES 39

1 INTEGRATED PEST MANAGEMENT

1.1 CONCEPT OF INTEGRATED PEST MANAGEMENT

It is more than fifty years since the integrated control concept was introduced as a theory of integrated pest management (IPM) and it continues to work well, in theory and practice to-day (Stern et al., 1959). Some novel ideas were described in the initial IPM concept, including the economic injury level, the economic threshold, the implementation of sampling plans for the prediction of pest occurrence, the use of selective insecticides and the recognition of ecosystem-level interactions between pests and their natural enemies (Jones et al., 2009). These progressive ideas, which are still widely accepted by producers, integrated two of the pest control methods available at that time, biological and chemical management systems (Castle et al., 2009) (Jones et al., 2009). This is not merely a combination of biological and chemical methods but an integration of all effective techniques for controlling the pest with the natural factors that regulate and limit their populations in the ecosystem (Coscollá, 2004). Some advances have been made in the integration of these two methods, especially the discovery of the chemical structure of insect pheromones, which has enabled their formulation into lures, and also the improvements in monitoring programs in the technology of monitoring (Jones et al., 2009).

Integrated production (IP) was originally defined by the International organization for biological and integrated control of noxious animals and plants (IOBC) as "*a farming system that produces high quality food and other products by using natural resources and regulating mechanisms to replace polluting inputs and to secure sustainable farming*" (Boller et al., 2004). IPM is not only a part of integrated fruit production but the main driving force of IP programs, focusing on arthropod pests, pathogens and weed management. Eight general principles have been identified for IPM and these were recently included in the Directive on the sustainable use of pesticides, which makes use of the following structure compulsory by 2014: (1) Measures for prevention and/or suppression of harmful organisms, (2) Tools for monitoring, (3) Threshold values as a basis for decision-making, (4) Non-chemical methods to be preferred, (5) Target-specificity and minimization of side effects, (6) Reduction of use to minimum necessary levels, (7) Application of anti-resistance strategies and (8) Records, monitoring, documentation and checks on success (EC, 2009).

Although IPM concepts were recognised in the late fifties, implementing them proved to be a problem because of their non-homogeneous requirements and apparently inadequate economic benefits (Freier and Boller, 2009). IPM programs are developing processes that must be regularly re-evaluated in order to improve their efficiency and incorporate changes in technology; environmental, health and safety requirements; and cultural, climatic and economic realities (Jones et al., 2009). IPM applies these principles to agricultural production in an attempt to reconcile demands for quality, profitability and respect for the environment: integrated control, fertilization, irrigation, pruning, etc. (Coscollá, 2004).

The development of an effective IPM model has resulted in it being accepted as a plant protection strategy for sustainable farming in each of the European countries, although its implementation is voluntary. Nowadays, IPM is commonly used for perennial crops, such as fruit and grapes (Freier and Boller, 2009) and for vegetables (Albajes et al., 1999).

Although there is a European directive for Organic Production, there is none for IP (Malavolta and Avilla, 2008). However, there is a non-legal document on standards and guidelines for food safety and integrated production at the European level (Boller et al., 2004) (EC, 2009). Chapter IIX of this document covers integrated plant protection methodology and states that preventive measures and observations on the status of pest, disease and weeds at the field level must be considered before intervention with direct plant protection measures. Important topics covered in relation to agrochemicals include choice of direct plant protection methods, recording of the characteristics of the application and pesticides used, storage and handling of chemical products, the importance of the spraying equipment, how to manage the disposal of surplus pesticides and legislative and/or food market requirements relating to pesticide residue analyses (Boller et al., 2004).

1.2 IPM AND IFP IN SPAIN

All agricultural regions of Spain have developed an IP system run by the local authorities (Sessler et al., 2004). The *Royal Decree 1201/2002* (BOE, 2002), established a National commission of integrated production to regulate the IP of agricultural products throughout Spain. This decree defined international criteria to be used to guarantee consumers' health, conserve the environment and ensure sustainable agriculture (Cobos, 2009).

In 2009, Spain registered a record 472,398 ha under IP, of which 40,273 ha were fruit-trees. Although the area employing IP methods in Catalonia is only 5.6% of the Spanish total, it runs to 30,947 ha and includes 1,822 growers or operators (Gencat, 2009c) (Mapa, 2009).

Counting both IP and conventional production, Spain is the fourth largest producer of peach and nectarines in the world and second largest in Europe (Euroresidentes, 2010). Government data from 2008 shows that Catalonia produces 28% of all Spanish peaches and is the main producer of apples for immediate consumption, 63.5% of the Spanish total (Mapa, 2010a). In 2009, 18.7% of all fruit-trees in Spain were under IP and 21.2% in Catalonia, where stone fruit-trees and pome fruit-trees cover 24 and 20%, respectively of the total IP surface (Gencat, 2009c) (Mapa, 2009).

Although in the three years from 2007 to 2009 the registered area of stone fruit-trees and pome fruit-trees under IP in Catalonia was lower than at any other time in the decade, during 2009, in Girona (where IPM was implemented in fruit orchards in the 80's) (Batllori et al., 2005), 2,139 ha were registered as IP pome fruit-trees (1,988.44 ha of apples) and 148 ha as IP stone fruit-trees (113.40 ha of peaches) (Mapa, 2009). Nevertheless, IPM is probably being used currently in a larger area than that registered under IP labels.

1.3 PESTS IN FRUIT-TREES FROM CATALONIA

The most damaging pests in Catalonia's pome fruit-trees are *Cydia pomonella* (Linnaeus), *Zeuzera pyrina* (Linnaeus), the aphids *Dysaphis plantaginea* Passerini, *Aphis pomi* (De Geer) and *Eriosoma lanigerum* (Hausmann), and in the case of pear trees also *Cacopsylla pyri* (Linnaeus). The most damaging stone fruit-trees pests are *Anarsia lineatella* Zeller, *Myzus persicae* (Sulzer), *Taeniothrips meridionalis* Priesner and *Thrips major* Uzel. Some pests are common to both kinds of fruit-trees, such as *Grapholita molesta* (Busk) and *Panonychus ulmi* (Koch) and in apple trees, peach trees and some pear varieties *Ceratitis capitata* (Wiedemann) is also a common species.

2 IMPORTANCE OF *CERATITIS CAPITATA*

The Tephritidae family comprises roughly 4,000 species and of these insects, about 1,400 species are known to develop in ripening fruits, including many commercial ones. Around 250 species are known to attack fruits that are either grown commercially or harvested from the wild. Although they are commonly known as 'fruit flies', larval development can occur in other parts of the host plants, such as flowers and stems. The wings of these flies are covered with patterns of variable size globally distributed along the wing (White and Elson-Harris, 1992).

The genus *Ceratitis* is endemic to tropical Africa (also known as the Afrotropical Region, in the Southern Sahara) and contains about 65 species, the majority of which are highly polyphagous (White and Elson-Harris, 1992).

One species of this genus is *C. capitata*, also known as 'The Mediterranean fruit fly' or 'medfly'. It is the most widespread member of the Tephritidae family (White and Elson-Harris, 1992), with a worldwide distribution and has been recorded in 132 countries and groups of islands in Africa, Asia, Central America-Caribbean, Europe, North America, Oceania and South America (Commonwealth-Institute-of-Entomology, 1984) (CAB-International, 1999).

Recent research regarding phylogeny, biogeography, host plant range and abundance of the medfly and its congeners within the subgenus *Ceratitis*, all support the view that they originated in Eastern Africa but molecular evidence contradicts this hypothesis, suggesting that a West African origin should also be considered (De Meyer et al., 2002). The first citation of medfly in Spain was in 1772 when it was described as a “mouche à dard” that destroyed large quantities of fruits around Grassé (Meridional coast of France) (Ros, 1988). The medfly was introduced to Australia from Europe around 1897 and after 1901 it appeared in Brazil, from where it gradually spread Northwards as far as Mexico (Bergsten et al., 1999).

The medfly is a highly adaptative polyphagous tropical fruit fly (Papadopoulos et al., 1996) which attacks more than three hundred and fifty botanical species from sixty five different families (Weems, 1981) (Liquido et al., 1991). The most vulnerable family is Rosaceae which is the main species cultivated in the Girona fruit area, with pome and stone fruit-trees. In this fruit area, the most susceptible hosts are peach (*Prunus persica* (Linnaeus)) varieties harvested between July and September, and apple (*Malus domestica* (Borkh)) and pear (*Pyrus communis* Linnaeus) varieties harvested between August and November. This last period coincides with the maximum population level detected in the Girona area (Escudero-Colomar et al., 2005) (Escudero-Colomar et al., 2008).

The medfly is considered to be one of the world’s most destructive fruit pests because of its global distribution, its wide range of hosts and its rapid dispersion due to expansion of world trade and travel (including personal luggage and mail transport), increased trade in agricultural produce, cultivation of medfly host plants in close proximity to human habitats, immigration and subsequent maintenance of certain cultural ties, customs and foods, smuggling of prohibited fruits and vegetables and increased vehicular traffic in medfly host plant growing areas (Bergsten et al., 1999).

3 TAXONOMY OF *CERATITIS CAPITATA*

Over time, medfly has had several different synonyms (White and Elson-Harris, 1992) (ITIS, 2010): *Tephritis capitata* Wiedemann (1824), *Trypeta capitata* (Wiedemann) (1824), *Ceratitis citriperda* MacLeay (1829), *Ceratitis hispanica* De Brême (1842) and *Pardalaspis asparagi* Bezzi (1924).

Nowadays, the taxonomic hierarchy of medfly is (Norrbom, personal communication):

Class:	Insecta
Order:	Diptera
Suborder:	Brachycera
Infraorder:	Muscomorpha (or Cyclorrhapha)

Family: Tephritidae
Subfamily: Dacinae
Tribe: Ceratitidini
Genus: *Ceratitis*
Subgenus: *Ceratitis*
Species: *Ceratitis capitata* (Wiedemann), 1824

4 BIOLOGY AND OTHER CHARACTERISTICS OF MEDFLY

Medfly is an insect with a complete metamorphosis or holometabolism, consisting of four stages: egg, larvae, pupa and adult. This species begins its life cycle when the adult female pierces the skin of fruits and vegetables and lays from 1 to 10 eggs. The eggs hatch and develop into larva which feed on the fruit pulp. There are three different stages of larvae (L_1, L_2 and L_3). Decaying and infested fruit usually falls to the ground when the maggots leave the fruit and burrow into the ground to pupate. Adult medflies emerge from the ground and mate, thus completing the cycle (CDFA, 2003).

The eggs of medfly are 1 mm x 0.2 mm, curved, shiny white when recently oviposited and later on yellowish (Ros, 1988). The threshold of egg development occurs at 11ºC (Shoukry and Hafez, 1979). The egg stage lasts two days (Boller, 1985).

The first larva instar is 2 mm and the third has an average length of 6.5 - 9 mm and width 1.2 - 1.5 mm (White and Elson-Harris, 1992). These apoda larva destroy pulp fruit with their chewer buccal system (Domínguez, 2007). The zero point of larval development occurs at 5ºC (Shoukry and Hafez, 1979) and the entire larva stage lasts 7 - 8 days (Boller, 1985).

The pupa is cylindrical, 4 - 4.3 mm long and dark reddish brown. Pupal stage takes place at temperatures between 22ºC and 30ºC, with 35ºC being fatal. At 60% R.H. the threshold of pupal development is 13ºC (Shoukry and Hafez, 1979) and this stage lasts 9 - 10 days (Boller, 1985). Other studies demonstrate that at 26ºC the pupal stage lasts 10 - 11 days and if the conditions are not favourable because of the low temperatures, this stage can be extended by several days (Mavrikakis et al., 2000). On the basis of this knowledge and in order to study the survivorship of pupa stage in Girona fruit growing area under winter conditions, chapter I of this Ph.D. was designed.

Adult medflies have an average body length of 4 mm and a wing length of 4.1 mm (De Meyer, 2000). They are black and white with a yellow abdomen and yellow marks on the thorax and their wings are banded with yellow (Bergsten et al., 1999). Both sexes can be easily separated from all other members of the family: the adult males have a black pointed expansion at the apex of the anterior pair of orbital setae and the females have a characteristic yellow wing pattern and the apical half of the scutellum is entirely black (White and Elson-Harris, 1992). Under laboratory conditions, males live an average of 36 days at 25ºC, while at the same temperature female longevity is 31 days, and longer when reared without males (67 days). The mating process begins with lekking behaviour, when male adults emit a pheromone and agitate their wings for 10 - 15 minutes. When a female approaches the male, she goes around him, he moves his head and flaps his wings, jumps on her and begins the copula (Field et al., 2002). It has been reported that the adult stage can survive the winter conditions from Valencia (Del Pino, 2000) and chapter I of this Ph.D. was designed also with the aim of studying their ability to survive in the Girona area under cooler conditions.

The preoviposition period lasts 4 - 6 days (Boller, 1985) and it has been recorded 826 eggs/female reared with males and 248 eggs/female if reared without them (Shoukry and Hafez, 1979).

5 DAMAGE AND ECONOMIC IMPORTANCE

Damage by medfly is caused by mated females which make a superficial scar on host fruits when they pierce the skin with their ovipositor to lay eggs and also by larvae that feed on the fruit pulp (Figures 1, 2 and 3). The tunnels produced by larval feeding allow the entry of secondary pathogens which destroy the fruit (Bergsten et al., 1999).

Figures 1, 2 and 3. Medfly female ovipositing, third-instar larvae emerging from a peach and inside view of a damaged fruit. Photos: E. Peñarrubia and M. Vilajeliu.

A high quantity of larvae can appear in a single fruit so the damage caused by medfly can be very severe. Direct damage is caused by the eggs which subsequently develop into larvae, which puncture the fruit and induce oxidation which prematurely decays the fruit and destroys it. Peaches also suffer serious damage, the pulp becomes soft, eventually acquiring an almost liquid consistency (Ros, 1988). Indirect damage induced by medfly stems from the decomposition of vegetal tissues due to the introduction of secondary organisms such as bacteria and fungi.

Over the past half century, Spanish citrus and fruit-trees have been damaged to varying degrees by *C. capitata*, depending on the area, time of year and climatic conditions (Sastre et al., 1999) (Alonso-Muñoz and García-Marí, 2004) (Martinez-Ferrer et al., 2006) (Escudero-Colomar et al., 2008). One region where research is currently been performed is the province of Girona, in the extreme North-East of Spain.

Owing to several factors, including fruit maturity, sensibility of the variety and climatic factors, it is difficult to correlate the population level of medfly with levels of damage encountered. It is, therefore, recommended that checks be made on fruit starting one month before harvest including all orientations of the fruit-trees, and at least 1,000 fruits per hectare in citrus (Navarro-Llopis and Femenia, 2004) or 500 by hectare in other less preferred hosts, such as apples (Escudero-Colomar personal communication).

Medfly is a quarantine species and host fruits and countries where it is established are forced to follow strict protocols imposed by importing countries in order to avoid the spread of the pest to new areas. Such protocols incur costs related to surveillance and monitoring of plots, chemical treatments, post-harvesting treatments and quarantine systems (Chueca, 2007).

6 CURRENT AND UNDER DEVELOPMENT CONTROL METHODS

Pest control methods must be economically viable, respectful of the environment and compatible with IP principles (Castañera, 2003). New economic evaluations of alternative methods of controlling the medfly must also be performed, as in 1997 (Enkerlin and Mumford, 1997), in order to identify the most effective techniques.

In recent years the most common pest control technique has been agrochemical, although new methodologies have been devised over the past years.

Absence or minimal quantities of toxic residues on fruits and the ecological benefits to the area enhance the advantage of IPM (Ros et al., 2002) over conventional chemical controls. Alternative strategies include botanical insecticides, biological control, microbial control, nematodes entomopathogens, photoinsecticides, attract and sterilize, attract and kill, sterile insect technique, incompatible insect technique using microflora, food based attractants and mass trapping technique, agronomical measures and post harvesting control.

6.1 CHEMICAL CONTROL

Over the last few decades insecticide has been the main control method for the suppression of medfly. The target stage is the adult, because eggs, larvae and pupae are protected in shelter sites, inside fruits and in the ground. Chemical treatments were based mainly on organophosphates and pyrethroids that have to be applied frequently, especially close to the harvest period.

One of the current trends in pesticide application is the development and use of pest thresholds. The economic threshold in IPM pome and stone fruits where mass trapping technique is not used is one capture in two consecutive controls inside pheromone or food attractants traps (with trimedlure or three component diffusers, respectively) or the presence of damage in fruits (Gencat, 2009a) (Gencat, 2009b).

The application of the European directive 91/414 EEC on pesticide marketing presupposes the withdrawal of numerous active ingredients (EEC, 1991). Some substances are currently authorized against medfly in Annex I of this normative (Table 1) (Gencat, 2009a) (Gencat, 2009b) (IRAC, 2010).

Table 1. Identification, safety period and uses of the active ingredients authorized for use in pome and stone fruit-trees against medfly.

GROUP AND IRAC MODE OF ACTION	CHEMICAL SUBGROUP	ACTIVE INGREDIENT	SAFETY PERIOD (DAYS)	REGISTERED IN SPAIN FOR	
				POME FRUIT-TREES	STONE FRUIT-TREES
(1) Acetylcholine esterase inhibitor	1B Organo-phosphates	Chlorpyrifos-methyl	15		X
(3) Sodium channel modulators	3A Pyrethroids	Deltamethrin	7	X	X
		Etofenprox	7		X
		Lambda cyhalothrin	7	X	X
(15) Inhibitors of chitin biosynthesis, type 0	Benzoylureas	Lufenuron	-	X	X

It is not anticipated that new active ingredients with high efficacy against medfly (Torà, 2008), will appear in the near future because currently, no new molecule is under development (Torné, 2008), although some substances such as Spinosad, would be registered for use in pome and stone fruits.

6.1.1 Spinosad

The bacterial species *Saccharopolyspora spinosad* sp. nov., from the actinomycetes group (Mertz and Yao, 1990) has been shown to produce two compounds with insecticidal properties, spinosyns A and D (Burns et al., 2001). Mixing the two spinosyns produces spinosad, derived naturally from the bacterium through fermentation (Vogt, 2004), an active ingredient that has been shown to be effective against several insects, including Diptera order (Burns et al., 2001). It acts as a stomach and contact poison though the activation of the nicotinic acetylcholine receptor, is the latest mechanism between the insecticides and belongs to the group 5 of the IRAC mode of action (Salgado, 1998) (Domínguez, 2007) (IRAC, 2010). Spinosad bait spray solution has been shown to be effective at field level for up to a week (Peck and McQuate, 2000).

Although spinosad has some advantages, quick degradation in the environment (Burns et al., 2001) and bee friendliness, due to the fact that they do not feed on it (Mangan and Moreno, 2009), it also has a disadvantage related to the production of honeydew (Chueca, 2007). After spinosad treatments in citrus orchards in Valencia, it was found that the black spots produced by other citrus pests such as aphids and whitefly species were more abundant, but it is thought that it is due to a problem associated with the bait (Chueca, 2007).

Some results show that bait spray is compatible with certain parasitoid species (Stark et al., 2004) (Piñero et al., 2009) but others suggest that spinosad can cause mortality among parasitoid wasps within 24 h of exposure (Michaud, 2003). This product is known to be rather selective to many predators, although in a field study performed on apple orchards spinosad resulted in reduced numbers of some arthropods groups (Vogt, 2004).

The commercial product is Spintor cebo (GF-120) and it is currently registered in Spain for use in citrus and olive orchards for the control of *C. capitata* and *Bactrocera oleae* (Gmelin), respectively (Mapa, 2009). Currently it is undergoing registration for use against medfly with stone fruits and it is not registered, nor is it being registered for use with pome fruits (Torné, 2008). This technique has also been used against other fruit flies, such as *Bactrocera invadens* Drew, Tsuruta & White and *Ceratitis cosyra* (Walker) (Vayssieres et al., 2009).

The most efficient system utilized against medfly seems to be on spot or banded sprays on trees, at low volume and big size of drops (Vergoulas and Torné, 2003), although it has also been tested on bait stations (Mangan and Moreno, 2007).

A tendency towards spinosad resistance has been observed in areas where it is used extensively, especially in California, where, since 1998, it has been the only insecticide used against *B. oleae* (Kakani et al., 2008) (Kakani et al., 2010), although in Hawaii tests on *Bactrocera dorsalis* (Hendel) for spinosad resistance produced negative results (Chou et al., 2010).

Another unwanted aspect of the use of spinosad is the phytotoxicity found on citrus fruits and sweet cherry foliage (*Prununs avium* Linnaeus) (Chueca, 2007) (DeLury et al., 2009). However, phytotoxicity induced by the bait could be reduced in the sweet cherry foliage through adaxial (upper) application and the use of lower concentrations on the abaxial (lower) surface (DeLury et al., 2009).

6.2 CONTROL WITH BOTANICAL INSECTICIDES

6.2.1 *Cestrum parqui*

The efficacy of aqueous extracts from *Cestrum parqui* L'Héritier (Solanaceae) has been tested in different stages of medfly development. These extracts showed a high toxicity to neonate larvae when ingested through diet. Pupation was inhibited at concentrations above 0.6%, larval development was delayed and there was a reduction in the percentage of pupae and adult emergence at lowered levels. The surviving adults had a diminished reproductive potential which had a negative effect on the offspring (Zapata et al., 2006).

6.2.2 *Citrus aurantium*

Crude or partially purified petroleum ether peel extract of *Citrus aurantium* Linnaeus has proved toxic to several fruit flies, including *C. capitata* and *B. oleae*. The chemical characterization of the active compounds is currently under study (Siskos et al., 2009).

6.2.3 *Citrus limon*

Mixing some of the constituents of *Citrus limon* (Linnaeus) peel with additional amounts of citral, 5.7-dimethoxycourmarin, and linalool might be useful as a natural insecticide for treatment of larvae and adults of medfly at specific concentrations (Salvatore et al., 2004).

6.2.4 *Solanum gilo*

Solanum gilo Raddi is originally from Africa and its fruits are edible. The effect on mortality and the delay of insect development were evaluated with an artificial diet treated with crude extracts of *S. gilo* (red fruits, green fruits, stems and leaves separately) at different concentrations. Mature extracts (from red fruits) at all concentrations showed major biological activity producing developmental delay in the period between neonate larvae and adult stages. At the highest concentration, these same extracts produced larval mortality at levels close to 100% (Bado et al., 2005).

6.2.5 *Thymus capitatus*, *Thymus herba-barona* and *Cinnamomum zeylanicum*

Studies have been made of essential oils with toxic effects on adult medflies: *Thymus capitatus* (Linnaeus)*, Thymus herba-barona* Loiseleur-Deslongchamps and *Cinnamomum zeylanicum* Nees. Medfly adults fed for three days with formulations containing a concentration of 1% of each of these three essential oils resulted in over 90% mortality after 72 hours. During the study, flies showed anomalous behaviour few hours after exposure (poor coordination, difficulty in flying, repeated and incoherent movement and motor deficiency), which indicates that the first consequences of ingesting even small quantities of the essential oils produces a negative effect on the nervous system (Passino et al., 1999).

6.3 BIOLOGICAL CONTROL

6.3.1 Parasitoids

Although medfly have been present in the Mediterranean area for a long time, there are no records of indigenous parasitoids in Spain (Papadopoulos and Katsoyannos, 2003) (Beitia et al., 2007) (Beitia et al., 2008). New research of parasitoids should now be carried out in Spanish orchards and the results compared with the findings of studies already conducted in other parts of the world (Ovruski et al., 2009) (Falcó et al., 2010).

In the Girona fruit growing area, along the development of the present work, the generalist parasite or hyperparasite *Melittobia acasta* (Walker) was found on medfly pupae (unpublished data). This ectoparasitoid is the only species of this genus native to Europe and has been reported on 28 groups of species as hosts, including some Diptera, although any of them is a fruit fly (Gonzalez et al., 2004a) (Gonzalez et al., 2004b). However, under laboratory conditions other species from the same genus, *Melittobia digitata* Dahms have been found to attack pupae from the fruit fly *Anastrepha ludens* (Loew) (González et al., 2008). With a view to establishing the importance of this species in medfly control, it would be necessary to conduct exhaustive screening tests, and to carry out studies of its abundance and parasitism level in the field.

Some exotic parasitoids have been studied in an attempt to control medfly in Spain: the larval-pupal parasitoid *Diachasmimorpha tryoni* (Cameron), the egg-pupal parasitoid *Fopius arisanus* (Sonan) and the two larval endoparasitoid *Diachasmimorpha longicaudata* (Ashmead) and *Psyttalia* (*Opius*) *concolor* (Szépligeti), all of them Hymenoptera of the Braconidae family (Alonso-Muñoz et al., 2008) (Beitia et al., 2008). Further research on these species is needed, including an assessment of the parasitoid/host larvae proportion and host exposure time if it is going to be devised an efficient mass rearing system for the parasitoids (Paranhos et al., 2008). Other factors including those which affect their flight, must be studied in order to determine the optimal conditions under which augmentive releases might succeed (Jang et al., 2000) (Moretti and Calvitti, 2003) (Wang and Messing, 2003) (Rousse et al., 2009).

Although the percentage of individual parasited is an important factor in the evaluation of the effectiveness of the parasitoids as a control agent for medfly, mortality levels caused by unsuccessful parasitoid attacks (oviposition attempts which then result in failure of the host egg development) must be also taken into account (Harris and Bautista, 2001) (Baeza-Larios et al., 2002).

Rates of parasitism usually vary between 30 and 70% (Hoffmeister, 1993), although in coffee plantations in Guatemala, levels of parasitism as high as 84% have been observed after aerial releases of *D. tryoni* (Sivinski et al., 2000). At low levels, parasitoids alone may not be enough to control temperate fruit flies but they might be a useful component in an IPM program (Hoffmeister, 1993).

New methodologies are being developed using PCR multiplex which make it possible to determine the parasitoid species and their parasitism rate (San-Andrés et al., 2009).

6.3.2 Predators

Screening research performed in Spain identified eighteen predator species from eleven different families (Monzó et al., 2007). In this research, it was observed that in the warmer months of the year, ants were the largest of all medfly predators; meanwhile in the colder season, spiders, *Staphylinidae* and other predators were more abundant (Urbaneja et al., 2006). Predators found were active at different seasonal periods, different moments of the day and they attacked different biological states of medfly living in the soil, thus ensuring the predation action over the whole year (Monzó et al., 2009b).

The wolf spider is a general predator, *Pardosa cribata* Simon, which preys on adults and third-instar larvae of medfly (Monzó et al., 2009a). Another spider predator is the female common sac spider *Chiracanthium mildei* L. Koch, which is a nocturnal predator of medfly males (Kaspi, 2000).

6.4 MICROBIAL CONTROL

The microbial control methods use beneficial organisms to maintain the population below the acceptable economical damage level. Over the past decade, studies have been made of the effectiveness of other microbial control agents on a number of Tephritid fruit flies (Dimbi et al., 2004) (Daniel, 2008) (Dimbi et al., 2009). Some entomopathogenic fungi have been successfully used against medfly using spray, bait or attract and kill techniques, and recent studies have shown their potential to control pupa when applied to the base of fruit-trees (Garrido-Jurado et al., 2009).

6.4.1 *Bacillus thuringiensis*

Toxicity bioassays against medfly have been carried out for several strains of the bacteria *Bacillus thuringiensis* (Berliner), recording maximum mortalities of only 30 - 40% (Vidal et al., 2008), although more studies are being undertaken in order to improve their lethal effect through the activation of protoxins (Vidal et al., 2009).

6.4.2 *Beauveria brongniartii* and *B. bassiana*

Evaluation of the virulence of fungi *Beauveria brongniartii* (Saccardo) and *B. bassiana* (Balsamo-Crivelli) identified mortality rates of 97.4 and 85.6%, respectively in medfly adults (Konstantopoulou and Mazomenos, 2005).

The effectiveness of a bioinsecticide based on the fungus *B. bassiana* has been tested on medfly in the laboratory and at field level, and was shown to reduce adult populations and protect citrus fruits (Ortu et al., 2009). Another study performed on citrus seedlings in a greenhouse, demonstrated a pre-pupal control efficiency of 66.6% (Almeida et al., 2007).

6.4.3 *Metarhizium anisopliae*

The *Metarhizium anisopliae* (Metschnikoff) fungus is pathogenic to medfly pre-pupae (Almeida et al., 2007). An extract of this fungus resulted in a mortality rate of around 90% at a concentration of 25 mg/g of diet, and reduced the fecundity and fertility of treated females by 94 and 53%, respectively (Castillo et al., 2000). The autodissemination technique is currently under research, because it could be a component of other strategies for the control of medfly such as bait spray and SIT (Dimbi et al., 2003) (Dimbi et al., 2009).

6.4.4 *Mucor hiemalis*

Feeding and contact bioassays using metabolites secreted by the fungus *Mucor hiemalis* Wehmer revealed high toxicity against the adult medfly and *B. oleae* (Konstantopoulou and Mazomenos, 2005) (Konstantopoulou et al., 2006).

6.5 CONTROL WITH NEMATODES

The pathogenicity of several entomopathogenic nematodes has been studied against larvae and pupae of medfly.

6.5.1 *Heterorhabditis* spp.

The pathogenicity of the nematode *Heterorhabditis* spp. (isolate IBCBn 05) has been evaluated against the pre-pupal stages of medfly, and was found to be effective at the concentration of 200 infective juveniles/medfly (Almeida et al., 2007).

6.5.2 *Steinernema* spp.

The level of parasitism achieved by the entomopathogen nematode *Steinernema* spp. has been studied at laboratory and field level, and has produced mortality levels of more than 50% in medfly larvae and pupae (Laborda et al., 2002).

6.6 CONTROL WITH PHOTOINSECTICIDES

This control is based on dye substances derived from the organic compound xanthene, which has been studied and tested as photoinsecticide on several dipteran species. The xanthene dye phloxine B acts as a photosensitizer. After having been ingested by an insect, if the specimen is exposed to light, its detoxifying systems are overwhelmed and it dies (Berni et al., 2009).

The effectiveness of the phloxine B at field level has been found to last up to one week and because the efficacy of the product depends on its ingestion, the formulation must be used as a bait (Peck and McQuate, 2000).

This product has shown acute light-dependent toxicity when larvae of medfly are exposed to light during the dispersion stage before pupation. Nevertheless, further research is required on this photoinsecticide before it can be used commercially (Berni et al., 2009).

6.7 CONTROL THROUGH ATTRACT AND STERILIZE

Insect grown regulators (IGR) used as chemosterilant agents are known as 3^{rd} generation insecticides and are used to control the population level of certain insects.

6.7.1 Lufenuron

Lufenuron bait is a phenyl-benzoylurea, chitin synthesis inhibitor (Bachrouch et al., 2008). It interrupts reproduction of medfly and prevents larvae hatching from eggs laid by females which have ingested it, or those which have mated with males that have eaten Lufenuron bait (Casaña-Giner et al., 1999). The effectiveness of this method is based on the horizontal transmission of sterility: by using the medfly’s capacity to find other individuals it is possible to sterilise a significant part of the population even though it has not ingested the bait (Casana-Giner et al., 1999), and it has a cumulative sterilizing effect on successive generations (Navarro et al., 2003).

In Spain Lufenuron (commercial product: MATCH, Syngenta), is currently registered as a spray for use against several pests (not medfly) and as a solid lure (commercial product: ADRESS, Lufenuron 3[RB], Syngenta) against medfly on several fruits (Mapa, 2010b).

Lufenuron applied must be sprayed as an emulsion in spots and its active life in the field can persist for at least two weeks but to maintain its effectiveness, it must be applied every fourteen days. The main advantage of this method is that it reaches a high percentage of the medfly population (Navarro-Llopis et al., 2004).

Solid bait can be utilised in delta traps containing Lufenuron with a proteinaceous gel (Navarro-Llopis et al., 2004) or in bait station that consists of a yellow plastic device containing a bait-gel based on Lufenuron 3%, a feeding stimulant and a tube containing attractants for male and female, acetate N-metil pirrolidina, which attracts males and females, ammonium acetate, which attracts females, and trimedlure, which attracts males (Bachrouch et al., 2008). This methodology has been reported to be successful in reducing the medfly population in Valencia (Navarro et al., 2007) and Mallorca Island (Alemany et al., 2008).

6.7.2 Other chemosterilant agents

Other IGR's have been shown to have a sterilizing effect when ingested, such as diflubenzuron, which reduced the fecundity of medfly (Sarasua and Santiago-Álvarez, 1983). The synthetic juvenile hormone analogue methoprene prevented adult medfly eclosion in laboratory tests, although at field level it was found to be ineffective (Saul et al., 1983). Cyromazine reduced fecundity and fertility and affected larval development of the medfly when it fed on the insecticide in drinking water (Budia and Viñuela, 1996). Triflumuron caused total suppression of egg hatch in a high concentration but to a lesser degree than lufenuron (Casaña-Giner et al., 1999).

6.8 CONTROL THROUGH ATTRACT AND KILL

In attract and kill technique, also named "lure and kill" or "bait stations", the insect is attracted by protein bait and subjected to a killing agent, which after a short period, eliminates the affected individuals. The application of a bait jointly with an insecticide for the control of fruit flies has been used since beginning of the 20th century (Chueca, 2007). This technique can be highly effective in controlling small, low-density, isolated populations (El-Sayed et al., 2009).

Fruit flies are strongly attracted to protein baits which can be used as a spot spray to the tree canopy at scattered points in the orchard or with bait station devices (ICMPFF, 2005). One of the advantages of the devices used in attract and kill methodology is that they do not saturate of flies and they need no maintenance (Navarro, 2009).

Currently, commercially available bait stations for the suppression of medfly belong to the second group described above, and are M3TM (Biagro S.L., Valencia, Spain). That specifically attracts medfly females and lasts for four months (Coltell, 2009) and magnet-med (Suterra España Biocontrol S.L., Cerdanyola del Vallès, Spain), lasts for three months (Torà, 2008). Other bait stations have been tested with promising results, using a modification of the Easy-trap® with a solution of sugar and methomyl on the outside (Ros et al., 2005b). New prototypes of bait stations for the control of several fruit flies are currently being developed and evaluated. These include spheres baited with ammonium salt and methomyl, killing bags, corn cobs, sponges and plastic bottles, all with protein bait and a killing agent (IAEA, 2008).

6.9 CONTROL THROUGH STERILE INSECT TECHNIQUE

6.9.1 Normal SIT

The sterile insect technique (SIT), included as an “autocidal” biological control method, is a technology that is currently applied against insects such as screw-worms, moths and fruit flies (Dyck et al., 2005).

The first SIT program used against medfly was conducted 40 years ago, since when it has been used successfully in several countries in order to prevent the appearance of medfly and to suppress or eradicate them (Rossler et al., 2000) (Klassen and Curtis, 2005).

This methodology is based on the mass rearing of male medfly, their sterilization using gamma radiation and their release from aircraft or ground vehicles into infested areas in order to compete with wild adults. When the sterile males find and mate with fertile females, they transfer their genetically modified sperm which produces infertile eggs, and thereby reduces the natural pest population (Knipling, 1955). It is considered to be the only nonchemical method capable of eradicating medfly (Bergsten et al., 1999). The recommended ratio used for optimal results is 100 sterile male medflies to 1 wild male medfly (Bergsten et al., 1999).

In Valencia, a mass-rearing and sterilization facility was inaugurated in 2007. Around 1,800 sterile males/ha are released every week in Valencian citrus groves (Generalitat-Valenciana, 2009). Different dosage levels are released in other areas. In Tunisia, for example, a mass-rearing and sterilization facility built in 2003, releases 1,000 sterile males/ha every week in the commercial area and another 2,000 sterile males/ha in the surrounding ‘buffer’ area to reduce the possibility of reinfestations (M'Saad Guerfali and Loussaief, 2008).

6.9.2 SIT without irradiation

Recent research has developed an alternative to the radiation based on reproductive sterility system. Known as transgenic embryonic lethality, this method results in complete (100%) embryonic mortality, without reducing their competitiveness to wildtype medfly in laboratory and field cage trials (Schetelig et al., 2009).

6.10 CONTROL THROUGH INCOMPATIBLE INSECT TECHNIQUE

Population suppression using incompatible insect technique (IIT) through microflora has been used successfully in the control of several insect species and is currently being studied as a means of controlling medfly. This approach is based on the mechanism of cytoplasmic incompatibility (CI) which is expressed as embryonic mortality in crosses between an infected male and a female of different infection status. *Wolbachia* spp. is a maternally inherited bacterium and intracellular manipulator of the insect's reproduction process, causing effects such as CI. It has been detected in several species of fruit flies of the genders *Bactrocera*, *Anastrepha*, *Rhagoletis* and *Ceratitis* (Arthofer et al., 2008) (Sapountzis et al., 2008).

Recent studies have been conducted on the *Wolbachia*-infected line of the genetic sexing strain used in SIT methodology. This transferred *Wolbachia* induces high levels of CI even after the temperature treatment required for the male-only production and can be used in cage population suppression tests similar to those used for SIT (Zabalou et al., 2009).

6.11 CONTROL THROUGH FOOD BASED ATTRACTANTS AND MASS TRAPPING TECHNIQUE

Initially, the attractants used against fruit flies were, liquid protein baits or fermented sugar substances (McPhail, 1939) but in recent years, new baits have been developed that are more effective and easier to manage. The first solid food-based synthetic attractants formulated in the mid nineties, consisted of ammonium acetate and 1.4 diaminobutane (putrescine) (Epsky et al., 1995). The effectiveness of these two components was augmented by the addition of the synergist trimethylamine presented in polyethylene membranes of slow liberation, which resulted in an increase in medfly captures (Ros et al., 1997) (Heath et al., 1997).

Similar substances have been tested as medfly attractants, for instance diaminoalkane (cadaverine) and putrescine which are considered to be equally efficient (Clemente-Angulo, 2002). Diaminoalkane has also been tested jointly with ammonium acetate and trimethylamine in three separated dispensers and compared with other attractants (Navarro-Llopis et al., 2008) and it is being used in seasonality studies (Escudero-Colomar et al., 2008) and mass trapping trials (II, III and IV chapters of this Ph.D.).

Research on the improvement of food-based synthetic lures for medfly enhanced the combination of the three compounds in only one lure. Diaminoalkane, ammonium acetate and trimethylamine were formulated in one dispenser as Ferag® CC D TM (SEDQ S.L., Barcelona, Spain) and putrescine, ammonium acetate and trimethylamine were also formulated in one dispenser as BioLure® Med Fly (Suterra S.L.) and Tripack® MFL (Kenogard S.A., Barcelona, Spain). Several studies have been carried out utilizing the attractants with only one dispenser to ascertain its effectiveness in comparison with the use of three dispensers (Alonso-Muñoz and García-Marí, 2007) (Lucas and Hermosilla, 2008).

Food baits developed for medfly attract mainly females and can directly reduce the numbers of pre-reproductive females, so they are a useful tool in fruit fly control (Lux et al., 2003). The same food baits are used to monitor the pest throughout the year, hanging at least one trap per field.

Mass trapping technique is a control system utilised against fruit flies (McPhail, 1939) (Steiner, 1952). The technique involves placing a high density of traps in the crop to be protected and achieves a measure of protection by removing a sufficiently high proportion of individuals from the population (Howse et al., 1998).

Using food based attractants in mass trapping in apple production has proved effective for both high and low medfly population levels (Escudero-Colomar et al., 2005). It has also been demonstrated to be an effective control method for citrus trees if used on low-density and isolated pest populations, though for higher populations it might be necessary to reinforce this process with chemical treatments (Putruele and Mouques, 2005) (Fibla et al., 2007) (Leza et al., 2008). Because the full potential of this fruit fly management technique has not yet been optimised for each fruit species, this thesis is examining the effectiveness of the technology as a means of protecting peaches.

It is important to emphasise that mass trapping has the major advantage of reducing pollution because less pesticide is used and it has limited contact with both fruit and the environment (Lux et al., 2003). Mass trapping also reduces the negative impact on beneficial entomofauna (Putruele and Mouques, 2005). A further advantage is that the attractants used have a minimum life of 120 days, which covers the entire ripening period of all relevant fruit varieties. Its main disadvantage is economic. In Spain, the current cost of mass trapping including labour, traps, attractants and insecticides amounts to 250 €/ha, corresponding to 5 €/trap (Navarro-Llopis et al., 2008). However, in recent years, the spraying of many insecticides has been forbidden in fruit orchards as a result of which, there are too few tools to fight the medfly. The development of other techniques is therefore essential if fruit production is to be properly protected.

The wide range of commercial complete equipments (systems composed by traps, attractants and insecticides) available in the market make necessary to test them to discover which are the most effective. Several trap models have been designed and tested, each with its own efficacy level, depending on factors such as the crop, the climatic conditions and the study area (Ros et al., 2005b) (Alonso-Muñoz and García-Marí, 2007) (Lucas and Hermosilla, 2008) (Navarro-Llopis et al., 2008). For the purpose of this thesis, the comparison of some material was carried out in the Girona fruit growing area and in other completely different setting, Réunion Island (II chapter of this Ph.D.), in order to compare their performance in two entirely different environments.

Currently, the use of insecticides in mass trapping technique presents a problem. An updating of the European Council directive 91/414 EEC relative to the pesticide marketing has recently proposed the withdrawal of the active ingredient dichlorvos or 2.2-dichlorovinyl dimethyl phosphate (DDVP) (EEC, 1991). Because of this prohibition, it is necessary to find another insecticide acceptable under the directive for use in mass trapping. After several trials in Girona, a suitable replacement appears to have been found, a concrete formulation of deltamethrin (III chapter of this Ph.D.). The positive effect of this insecticide has been studied by other authors (Alemany et al., 2005) (Ros et al., 2005a) (Ros et al., 2005b).

6.12 AGRONOMICAL MEASURES

Crop sanitation plays an important role in the reduction of the population at field level: the collection, burying or destruction of non commercial fruits, including those that are fallen, damaged or over-ripe is an essential part of IPM methodology. For example, the harvesting of non commercial fruits will eliminate those that are larvae-infested, thus removing suitable places for females to lay their eggs (Chueca, 2007). Similarly, in equatorial Africa, where small growers tend to harvest fruits before they ripen, this strategy avoids fruit infestation, rather than preventing it later by fruit fly management. Unripe fruits are either not yet infested or contain only the eggs or larvae from first instars, and although the ripe fruit is usually infested with fruit flies, it is utilised locally (Lux et al., 2003).

6.13 POST-HARVEST CONTROL

Several physical methodologies can be applied to post-harvest fruits and may be required to export hosts from medfly-infested areas that could support establishment of the pest (Torres-Rivera and Hallman, 2007).

6.13.1 Cold treatments

Cold treatments aim to reduce the survival level of the target species (Palou et al., 2007). Cold treatments which hold fruits at 1.1ºC, 1.7ºC and 2.2ºC for 14, 16 and 18 days, respectively are currently being used to disinfest tangerines shipped from Spain to United States and China. Spanish citrus fruits to be exported to Australia must remain at between 0ºC and 2.2ºC for a period of 10 and 16 days. Spanish citrus fruit exported to Japan must be kept at temperatures of less than 2ºC for a minimum period of 16 days; lemons, mandarins and oranges for 17 days (Palou et al., 2007) (Torres-Rivera and Hallman, 2007).

6.13.2 Warm treatments

Heat treatments, which can be dry or steam driven, are performed with the aim of exceeding the longest survival period of the species in these conditions

(Palou et al., 2007). Mangoes are immersed in water at 46.1º for 65 - 110 minutes before they are shipped, depending on their shape, mass and origin; And papayas are treated with hot air before shipment (Torres-Rivera and Hallman, 2007).

6.13.3 Artificial vision system

It is necessary to eliminate infested fruit through a non destructive inspection process before it arrives at the commercial classification line. In Valencia a multispectral and artificial vision system is used to identify the origin of the external damage in citrus fruit (Castañera, 2003). A recent study has been published describing the hardware system which uses three cameras in line to detect the smallest possible defects, such as insects bites in an apple (Zou et al., 2010).

6.13.4 Ionizing irradiation

Ionizing irradiation can be performed with γ rays or with X rays (Hallman, 1999). One of the advantages of this method is that it can be applied after packing and palletizing but it also has limitations, as it does not guarantee high medfly mortality, although afterwards, individuals are unable to development completely and reproduce. The minimum ionizing radiation dose to prevent adult emergence from medfly third-instars in papaya, orange, mandarin, mango, peach and grapefruit has been determined. Avocado has been cited as another possible fruit to be treated in this way (Torres-Rivera and Hallman, 2007) (Palou et al., 2007).

6.13.5 Insecticidal atmosphere

This quarantine method exposes fruit to varying periods of time in a controlled atmosphere, with modified levels of CO_2 and O_2, together with the application of heat or cold. Several studies performed in Valencia on mandarin and orange cultivars obtained positive results, utilizing 95 and 98% of CO_2, respectively (Palou et al., 2007).

6.14 COMBINATION OF SEVERAL CONTROL METHODS

Mass trapping combined with spinosad-based bait sprays are control components that are compatible with biological control and could be combined in an IPM system for *C. capitata*, as occurred in a field level trial of persimmon with adjacent coffee plantings (McQuate et al., 2005). Spinosad-based protein bait sprays utilized in foliar applications (either to all rows or to every fifth row), in combination with systematic field sanitation proved to be successful in reducing the population level of female *B. dorsalis* and the damaged fruits in papaya orchards (Piñero et al., 2009).

In a study of the feeding and foraging of medfly adults using spinosad bait, it was observed that protein-deficient flies were more active and found the bait more often than those that were protein-fed. This suggests that adding protein to the diet of male adults to be used in SIT would reduce their response to baits, thereby reducing their mortality. This would indicate the need for the simultaneous use of the spinosad bait sprays and the sterile insect technique (Barry et al., 2003).

Studies have been made of the possibility of combining two biological agents: SIT and biological control using parasitoids. Interactions between the capacity of sterile males and two species of parasitoids to suppress caged fly populations were tested, although further research is needed (Rendon et al., 2006). The methodology combining both techniques would require rearing the parasitoid *D. longicaudata* on a genetic sexing strain of medfly. This procedure has been proved not to produce any negative effect on several biological parameters studied (Viscarret et al., 2006). A recent study showed that the mass-rearing process of this parasitoid species appears to have induced the selection of more aggressive, fertile and precocious females. However, it was concluded that the process of adaptation to mass-rearing conditions did not influence foraging and ovipositional behaviours (Gonzalez et al., 2010).

7 REFERENCES

Albajes, R., Gullino, M.L., Van Lenteren, J.C., Elad, Y., 1999. Integrated Pest and Disease Management in Greenhouse Crops. Developments in Plant Pathology. Kluwer Academic Publishers, London, UK.

Alemany, A., González, A., Juan, A., Tur, C., 2008. Evaluation of the chemosterilization experiment against the Mediterranean Fruit Fly Ceratitis capitata (Wied.) (Diptera: Tephritidae) in the Sóller Valley (Mallorca, Balearic Islands, Spain). First Meeting of TEAM, Palma de Mallorca 7-8 April, 2008, pp. 83.

Alemany, A., Miranda, M.A., Vestergaard, F., Abdali, A., 2005. Deltamethrine as a replacement for diclorvos (DDVP) strips used as a retention system in fruit fly traps. Development of improved attractants and their integration into Fruit Fly SIT management programmes, Vienna 5-7 May 2005, pp. 11.

Almeida, J.E.M., Batista, A., Oliveira, F.C., Raga, A., 2007. Pathogenicity of the entomopathogenic fungi and nematode on medfly Ceratitis capitata (Wied.) (Diptera: Tephritidae). BioAssay 2**,** 1-7.

Alonso-Muñoz, A., Arilla, J., Torres, J., Fernández, L., García, E., García-Marí, F., 2008. Influence of nearby fig trees on medfly captures of traps located in citrus orchards. First Meeting of TEAM, Palma de Mallorca 7-8 April, 2008, pp. 53.

Alonso-Muñoz, A., García-Marí, F., 2004. Control de Ceratitis capitata (Diptera: Tephritidae) en cítricos utilizando trampeo masivo. Phytoma España 157**,** 28-37.

Alonso-Muñoz, A., García-Marí, F., 2007. Comparación de cuatro tipos de mosqueros comerciales de captura de hembras y machos de Ceratitis capitata (Diptera: Tephritidae). Boletín de Sanidad Vegetal, Plagas 33**,** 399-407.

Arthofer, W., Schneider, D., Krammer, M., Riegler, M., Miller, W.J., Stauffer, C., 2008. Genome dynamics of Wolbachia in Rhagoletis cerasi. First Meeting of TEAM, Palma de Mallorca 7-8 April, 2008, pp. 10.

Bachrouch, O., Mediouni-Ben, J., Alimi, E., Skillman, S., Kabadou, T., Kerber, E., 2008. Efficacy of the lufenuron bait station technique to control Mediterranean Fruit Fly (Medfly) Ceratitis capitata in citrus orchards in Northern Tunisia. Tunisian journal of plant protection 3**,** 35-45.

Bado, S.G., Linares, M.A., Clemente, S., Cirigliano, A., 2005. Determinación de la actividad insecticida de extractos obtenidos de Solanum gilo R. (Solanaceae) sobre Ceratitis capitata W. (Diptera: Tephritidae). Nutri-fitos 101**,** 73-80.

Baeza-Larios, G., Sivinski, J., Holler, T., Aluja, M., 2002. The ability of Coptera haywardi (Ogloblin) (Hymenoptera : Diapriidae) to locate and attack the pupae of the Mediterranean fruit fly, Ceratitis capitata (Wiedemann) (Diptera : Tephritidae), under seminatural conditions. Biological Control 23**,** 213-218.

Barry, J.D., Vargas, R.I., Miller, N.W., Morse, J.G., 2003. Feeding and foraging of wild and sterile Mediterranean fruit flies (Diptera : Tephritidae) in the presence of spinosad bait. Journal of Economic Entomology 96**,** 1405-1411.

Batllori, J.L., Vilajeliu, M., Creixell, A., Carbo, M., Garcia, N., Esteba, G., Raset, F., Vayreda, F., Gine, M., Curos, D., Cornella, J., 2005. Reduction of insecticide sprayings by using alternative methods in commercial apple orchards. Bulletin OILB/SROP 28**,** 83-88.

Beitia, F., Pérez-Hinarejos, M., Garzón, E., Santiago, S., Falcó, V., Castañera, P., 2008. Potential for biological control of Ceratitis capitata (Wiedemann) (Diptera:Tephritidae) by parasitoids in Spanish Mediterranean areas. First Meeting of TEAM, Palma de Mallorca 7-8 April, 2008, pp. 36.

Beitia, F., Pérez-Hinarejos, M., Santiago, S., Garzón, E., Tarazona, I., Falcó, J.V., 2007. Control biológico con parasitoides. Levante Agrícola 385**,** 145-150.

Bergsten, D., Lance, D., Stefan, M., 1999. Mediterranean fruit flies and their management in the U.S.A. The Royal Society of Chemistry 10**,** 207-212.

Berni, J., Rabossi, A., Pujol-Lereis, L.M., Tolmasky, D.S., Quesada-Allue, L.A., 2009. Phloxine B affects glycogen metabolism in larval stages of Ceratitis capitata (Diptera: Tephritidae). Pesticide Biochemistry and Physiology 95**,** 12-17.

BOE, 2002. (Boletín Oficial del Estado) nº287 del 30 de noviembre del 2002, Real Decreto 1201/2002, de 20 de noviembre, por el que se regula la producción integrada de productos agrícolas.

Boller, E.F., 1985. Rhagoletis cerasi and Ceratitis capitata. Handbook of Insect Rearing 2**,** 135-144.

Boller, E.F., Avilla, J., Joerg, E., Malavolta, C., F.G., W., Esbjerg, P., 2004. Integrated Production: principles and technical guidelines (3rd edition). IOBC/WPRS Bulletin, at http://www.iobc.ch. 27 (2).

Budia, F., Viñuela, E., 1996. Effects of Cyromazine on adult C. capitata (Diptera: Tephritidea) on mortality and reproduction. Entomological society of America 89, 826-831.

Burns, R.E., Harris, D.L., Moreno, D.S., Eger, J.E., 2001. Efficacy of spinosad bait sprays to control Mediterranean and Caribbean fruit flies (Diptera : Tephritidae) in commercial citrus in Florida. Florida Entomologist 84, 672-678.

CAB-International, 1999. Distribution maps of plant pests, Ceratitis capitata. Commonwealth institute of entomology. www.cabi.org/.

Casaña-Giner, V., Gandia-Balaguer, A., Mengod-Puerta, C., Primo-Millo, J., Primo-Yufera, E., 1999. Insect growth regulators as chemosterilants for Ceratitis capitata (Diptera : Tephritidae). Journal of Economic Entomology 92, 303-308.

Castañera, P., 2003. Control integrado de la mosca mediterránea de la fruta, Ceratitis capitata (Wiedmann) (Díptera: Tephritidea) en cítricos. Phytoma 153, 131-133.

Castillo, M.A., Moya, P., Hernandez, E., Primo-Yufera, E., 2000. Susceptibility of Ceratitis capitata Wiedemann (Diptera : Tephritidae) to entomopathogenic fungi and their extracts. Biological Control 19, 274-282.

Castle, S.J., Goodell, P.B., Palumbo, J.C., 2009. Implementing principles of the integrated control concept 50 years later - current challenges in IPM for arthropod pests. Pest Management Science 65, 1263-1264.

CDFA, 2003. Mediterranean fruit fly impact on you. http://www.cdfa.ca.gov/phpps/pdep/treatment/medfly_treatment.html.

Chou, M.Y., Haymer, D.S., Feng, H.T., Mau, R.F.L., Hsu, J.C., 2010. Potential for insecticide resistance in populations of Bactrocera dorsalis in Hawaii: spinosad susceptibility and molecular characterization of a gene associated with organophosphate resistance. Entomologia Experimentalis Et Applicata 134, 296-303.

Chueca, P., 2007. Mecanización de métodos de control de Ceratitis capitata (Wiedemann) en cítricos. Departamento de mecanización y tecnología agraria. Unversidad Politécnica de Valencia, Valencia, pp. 246.

Clemente-Angulo, S., 2002. Atrayentes alternativas para Ceratitis capitata. Universidad Politécnica de Valencia, Valencia.

Cobos, J.M., 2009. Una perspectiva de la Producción Integrada en el estado español. Phytoma España 202, 20-22.

Coltell, N., 2009. Trampas-cebo M3 para combatir la mosca de la fruta. Phytoma España 213, 46.

Commonwealth-Institute-of-Entomology, 1984. Distribution maps of pests, Map Nº1 Ceratitis capitata (Wiedemann).

Coscollá, R., 2004. Introducción a la Protección Integrada. Phytoma.

Daniel, C., 2008. Susceptibility on Rhagoletis cerasi to entomopathogenic fungi. First Meeting of TEAM, Palma de Mallorca 7-8 April, 2008, pp. 38.

De Meyer, M., 2000. Systematic revision of the subgenus Ceratitis MacLeay s.s. (Diptera, Tephritidae). Zoological Journal of the Linnean Society. 128, 439-467.

De Meyer, M., Copeland, R.S., Wharton, R.A., McPheron, B.A., 2002. On the geographic origin of the Medfly Ceratitis capitata (Wiedemann) (Diptera:

Tephritidae). 6th International Fruit Fly Symposium, 6-10 May 2002, Stellenbosch, South Africa, pp. 45-53.

Del Pino, A., 2000. Efecto de factores abióticos y edáficos naturales en el ciclo biológico de Ceratitis capitata (Wiedemann) (Diptera: Tephritidae). Universidad Politécnica de Valencia.

DeLury, N.C., Thistlewood, H., Routledge, R., 2009. Phytotoxicity of GF-120® NF Naturalyte® fruit fly bait carrier on sweet cherry (Prunus avium L.) foliage. Pest Management Science 65**,** 52-59.

Dimbi, S., Maniania, N.K., Ekesi, S., 2009. Effect of Metarhizium anisopliae inoculation on the mating behavior of three species of African Tephritid fruit flies, Ceratitis capitata, Ceratitis cosyra and Ceratitis fasciventris. Biological Control 50**,** 111-116.

Dimbi, S., Maniania, N.K., Lux, S., Ekesi, S., Mueke, J.K., 2003. Pathogenicity of Metarhizium anisopliae (Metsch.) Sorokin and Beauveria bassiana (Balsamo) Vuillemin, to three adult fruit fly species: Ceratitis capitata (Weidemann), C-rosa var. fasciventris Karsch and C-cosyra (Walker) (Diptera : Tephritidae). Mycopathologia 156**,** 375-382.

Dimbi, S., Maniania, N.K., Lux, S.A., Mueke, J.M., 2004. Effect of constant temperatures on germination, radial growth and virulence of Metarhizium anisopliae to three species of African tephritid fruit flies. Biocontrol 49**,** 83-94.

Domínguez, J., 2007. Estudio de la aplicación extensiva del método quimioesterilizante en el control de Ceratitis capitata (Wiedemann). Desarrollo y mejora de los sistemas de atracción. Departamento de ecosistemas agroforestales. Universidad Politécnica de Valencia, Valencia, pp. 140.

Dyck, V.A., Hendrichs, J., Robinson, A.S., 2005. Sterile Insect Technique. Principles and practice in area-wide Integrated Pest Management.

EC, 2009. Directive of 21 October 2009 concerning the sustainable use of pesticides (2009/128/EC).

EEC, 1991. Council Directive of 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC).

El-Sayed, A.M., Suckling, D.M., Byers, J.A., Jang, E.B., Wearing, C.H., 2009. Potential of "Lure and Kill" in long-term pest management and eradication of invasive species. Journal of economic entomology 112**,** 815-835.

Enkerlin, W., Mumford, J., 1997. Economic evaluation of three alternative methods for control of the Mediterranean fruit fly (Diptera: Tephritidae) in Israel, Palestinian territories, and Jordan. Journal of Economic Entomology 90**,** 1066-1072.

Epsky, N.D., Heath, R.R., Guzman, A., Meyer, W.L., 1995. Visual cue and chemical cue interactions in a dry trap with food-based synthetic attractant for Ceratitis capitata and Anastrepha ludens (Diptera: Tephritidae). Environmental Entomology 24**,** 1387-1395.

Escudero-Colomar, L.A., Vilajeliu, M., Batllori, L., 2005. Captura masiva para el control de la mosca mediterránea de la fruta (Ceratitis capitata (Wied.)) en manzano. Phytoma España, pp. 5.

Escudero-Colomar, L.A., Vilajeliu, M., Batllori, L., 2008. Seasonality in the occurrence of the Mediterranean fruit fly [Ceratitis capitata (Wied.)] in the north-east of Spain. Journal of Applied Entomology 132**,** 714-721.

Euroresidentes, 2010. Melocotón. www.euroresidentes.com/Alimentos/melocoton.htm.
Falcó, J.V., Verdú, M.J., Bolinches, J.V., 2010. Valoración cuantitativa y cualitativa de parasitoides en capturas por trampeo masivo de Ceratitis capitata. Phytoma España 221, 18-26.
Fibla, J.M., Martínez-Ferrer, M.T., Campos, J.M., Monfort, R., Colell, R., 2007. Control de Ceratitis capitata Wied en variedades tempranas de cítricos y pequeñas superficies, mediante diferentes estrategias de trampeo masivo. Levante Agrícola 385, 126-134.
Field, S.A., Kaspi, R., Yuval, B., 2002. Why do calling medflies (Diptera : Tephritidae) cluster? Assessing the empirical evidence for models of medfly lek evolution. Florida Entomologist 85, 63-72.
Freier, B., Boller, E.F., 2009. Chapter 14. Integrated Pest Management in Europe – history, policy, achievements and implementation. Integrated Pest Management: dissemination and impact., pp. 435-454.
Garrido-Jurado, I., Santiago-Álvarez, C., Quesada-Moraga, E., 2009. Efecto de la humedad y temperatura del suelo sobre la virulencia de hongos entomopatógenos frente a puparios de Ceratitis capitata (Wiedemann) (DIptera: Tephritidae). VI Congreso Nacional de Entomología Aplicada, Palma de Mallorca 19-23 Octubre, 2009, pp. 54.
Gencat, 2009a. Norma tècnica per a la producció integrada de fruita de llavor. www.produccióintegrada.cat.
Gencat, 2009b. Norma tècnica per a la producció integrada de fruita de pinyol. www.produccióintegrada.cat.
Gencat, 2009c. Producció Integrada - Catalunya. www20.gencat.cat.
Generalitat-Valenciana, 2009. Las actuaciones para el control de la mosca de la fruta reducen su incidencia en un 90% en el último año. http://www.agricultura.gva.es.
Gonzalez, J.M., Genaro, J.A., Matthews, R.W., 2004a. Species of Melittobia (Hymenoptera: Eulophidae) established in Bahamas, Costa Rica, Cuba, Hispaniola, Puerto Rico, and Trinidad. Florida Entomologist 87, 619-620.
Gonzalez, J.M., Teran, J.B., Matthews, R.W., 2004b. Review of the biology of Melittobia acasta (Walker) (Hymenoptera : Eulophidae) and additions on development and sex ratio of the species. Caribbean Journal of Science 40, 52-61.
Gonzalez, P.I., Montoya, P., Perez-Lachaud, G., Cancino, J., Liedo, P., 2010. Host discrimination and superparasitism in wild and mass-reared Diachasmimorpha longicaudata (Hym.: Braconidae) females. Biocontrol Science and Technology 20, 137-148.
González, J., Matthews, R., Bradleigh, S., 2008. Distribution and host records of Melittobia (Hymenoptera: Eulophidae) from Mexico. Revista mexicana de biodiversidad 79, 529-531.
Hallman, G.J., 1999. Ionizing radiation quarantine treatments against tephritid fruit flies. Postharvest Biology and Technology 16, 93-106.
Harris, E.J., Bautista, R.C., 2001. Implications of host mortality on the economics of Fopius arisanus (Hymenoptera : Braconidae) mass rearing. Biocontrol 46, 275-287.
Heath, R.R., Epsky, N.D., Dueben, B.D., Rizzo, J., Jeronimo, F., 1997. Adding methyl-substituted ammonia derivatives to a food-based synthetic

attractant on capture of the Mediterranean and Mexican fruit flies (Diptera : tephritidae). Journal of Economic Entomology 90, 1584-1589.

Hoffmeister, T., 1993. The parasitoid complexes of frugivorous Fruit Flies on Central Europe. Fruit Flies: Biology and Management, pp. 125-127.

Howse, P., Stevens, I., Jones, O., 1998. Feromonas de insectos y su uso en el control de plagas.

IAEA, 2008. Development of bait stations for fruit fly suppression in support of SIT. FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Mazatlán, Mexico, 30 October-1 November 2008, pp. 15.

ICMPFF, 2005. Integrated system approach for managing fruit flies. http://www.icmpff.org.

IRAC, 2010. Insecticide resistance action committee, eclassification. http://www.irac-online.org/eClassification.

ITIS, 2010. Integrated Taxonomic Information System: Ceratitis capitata (Wiedemann, 1824). www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=143196.

Jang, E.B., Messing, R.H., Klungness, L.M., Carvalho, L.A., 2000. Flight tunnel responses of Diachasmimorpha longicaudata (Ashmead) (Hymenoptera : Braconidae) to olfactory and visual stimuli. Journal of Insect Behavior 13, 525-538.

Jones, V.P., Unruh, T.R., Horton, D.R., Mills, N.J., Brunner, J.F., Beers, E.H., Shearer, P.W., 2009. Tree fruit IPM programs in the western United States: the challenge of enhancing biological control through intensive management. Pest Management Science 65, 1305-1310.

Kakani, E., Zygourides, N., Seraphides, N., Thompson, P., Zalom, F., Mathiopoulos, K., 2008. Low levels of spinosad resistance in the olive fruit fly in California. First Meeting of TEAM, Palma de Mallorca 7-8 April, 2008, pp. 63.

Kakani, E.G., Zygouridis, N.E., Tsoumani, K.T., Seraphides, N., Zalom, F.G., Mathiopoulos, K.D., 2010. Spinosad resistance development in wild olive fruit fly Bactrocera oleae (Diptera: Tephritidae) populations in California. Pest Management Science 66, 447-453.

Kaspi, R., 2000. Attraction of female Chiracanthium mildei (Araneae : Clubionidae) to olfactory cues from male Mediterranean fruit flies Ceratitis capitata (Diptera : Tephritidae). Biocontrol 45, 463-468.

Klassen, W., Curtis, C.F., 2005. History of the Sterile Insect Technique. In: Netherlands, S., (Ed.), Sterile Insect Technique. Principles and practice in area-wide Integrated Pest Management.

Knipling, E.F., 1955. Possibilities of insect control or eradicaction through the use of sexually sterile males. Jounal of Applied Entomology 48, 459-462.

Konstantopoulou, M.A., Mazomenos, B.E., 2005. Evaluation of Beauveria bassiana and B-brongniartii strains and four wild-type fungal species against adults of Bactrocera oleae and Ceratitis capitata. Biocontrol 50, 293-305.

Konstantopoulou, M.A., Milonas, P., Mazomenos, B.E., 2006. Partial purification and insecticidal activity of toxic metabolites secreted by a Mucor hiemalis strain (SMU-21) against adults of Bactrocera oleae and Ceratitis capitata (Diptera : Tephritidae). Journal of Economic Entomology 99, 1657-1664.

Laborda, R., Bargues, L., Navarro, C., Montoro, E., Llopis, E., Barajas, O., Arroyo, M., García, E.M., Martínez, A., Sayaguéz, J.M., 2002. Efecto de las aplicaciones de nematodos entomopatógenos para el control de la mosca del mediterráneo. Phytoma España 144**,** 160-161.

Leza, M.M., Romero, A., Capllonch, M., Ferreras, M., Alemany, A., 2008. Comparative results of insecticide bait spray vs mass trapping technique against the mediterranean fruit fly Ceratitis capitat (Wied.) (Diptera:Tephritidae). First Meeting of TEAM, Palma de Mallorca 7-8 April, 2008, pp. 61.

Liquido, N.J., Shinoda, L.A., Cunningham, R.T., 1991. Host plants of the Mediterranean fruit fly (Diptera: Tephritidae): an annotated world review. Entomological Society of America, Lanham, MD.

Lucas, A., Hermosilla, A., 2008. Eficacia de diferentes atrayentes y mosqueros para el control de mosca de la fruta (Ceratitis capitata) en uva de mesa. Agricola Vergel 27**,** 260-268.

Lux, S.A., Ekesi, S., Dimbi, S., Mohamed, S., Billah, M., 2003. Mango-infesting fruit flies in Africa: perspectives and limitations of biological approaches to their management. Biological Control in IPM Systems in Africa, 277-293.

M'Saad Guerfali, M., Loussaief, F., 2008. The Sterile Insect Technique for the control of Ceratitis capitata (Weideman) in oranges orchards in Beni Khaled, Tunisia. First Meeting of TEAM, Palma de Mallorca 7-8 April, 2008, pp. 78.

Malavolta, C., Avilla, J., 2008. Situación de la Producción Integrada en Europa. Phytoma España 202**,** 17-18.

Mangan, R., Moreno, D., 2007. Development of bait stations for Fruit Fly population suppression. Journal of Economic Entomology 100**,** 440-450.

Mangan, R.L., Moreno, A.T., 2009. Honey bee foraging preferences, effects of sugars, and Fruit Fly toxic bait components. Journal of Economic Entomology 102**,** 1472-1481.

Mapa, 2009. Producción Integrada. Estadística. www.mapa.es.

Mapa, 2010a. Estadística. Avances de superficie y producción. www.mapa.es.

Mapa, 2010b. Registro de productos fitosanitarios. www.mapa.es.

Martinez-Ferrer, M.T., Campos, J.M., Fibla, J.M., 2006. Population dynamics of Ceratitis capitata on citrus in northeast Spain: the influence of adjacent host fruit trees. Bulletin OILB/SROP 29**,** 77-84.

Mavrikakis, P.G., Economopoulos, A.P., Carey, J.R., 2000. Continuous winter reproduction and growth of the Mediterranean fruit fly (Diptera : Tephritidae) in Heraklion, Crete, southern Greece. Environmental Entomology 29**,** 1180-1187.

McPhail, M., 1939. Protein lures for fruitflies. Journal of Economic Entomology 32**,** 758-761.

McQuate, G.T., Sylva, C.D., Jang, E.B., 2005. Mediterranean fruit fly (Dipt., Tephritidae) suppression in persimmon through bait sprays in adjacent coffee plantings. Journal of Applied Entomology 129**,** 110-117.

Mertz, F., Yao, R., 1990. Saccharopolyspora spinosa sp. nov. isolated from soil collected in a sugar mill rum still. International journal of systematic bacteriology 40**,** 34-39.

Michaud, J.P., 2003. Toxicity of fruit fly baits to beneficial insects in citrus. Journal of Insect Science 3**,** 1-9.

Monzó, C., Molla, O., Castañera, P., Urbaneja, A., 2009a. Activity-density of Pardosa cribata in Spanish citrus orchards and its predatory capacity on Ceratitis capitata and Myzus persicae. BioControl 54**,** 393-402.

Monzó, C., Mollá, O., Montón, H., Urbaneja, A., Castañera, P., 2007. Artrópodos depredadores potenciales de Ceratitis capitata presentes en el suelo de los cítricos. Levante Agrícola 385**,** 152-156.

Monzó, C., Sabater-Muñoz, B., Catañera, P., Urbaneja, A., 2009b. Control biológico de conservación en los suelos de cítricos: Influencia sobre la mosca del mediterráneo Ceratitis capitata (Wiedemann) (Diptera: Tephritidae). VI Congreso Nacional de Entomología Aplicada, Palma de Mallorca 19-23 Octubre, 2009, pp. 57.

Moretti, R., Calvitti, M., 2003. Mortality by parasitization in the association between the egg-pupal parasitoid Fopius arisanus and Ceratitis capitata. Biocontrol 48**,** 275-291.

Navarro, V., 2009. La técnica de atracción y muerte: una apuesta de futuro para reemplazar el trampeo masivo. Phytoma España 213**,** 45.

Navarro, V., Domínguez, J., Sanchís, J., Primo, J., 2007. Aplicación del método quimioesterilizantes para el control de Ceratitis capitata (Wiedemann). Levante Agrícola 385**,** 177-180.

Navarro, V., Sanchís, J., Moya, P., Primo, E., 2003. La quimioesterilización como método de lucha contra Ceratitis capitata (Wied.). Fruticultura Profesional 137**,** 19-29.

Navarro-Llopis, V., Alfaro, F., Dominguez, J., Sanchis, J., Primo, J., 2008. Evaluation of traps and lures for mass trapping of Mediterranean fruit fly in citrus groves. Journal of Economic Entomology 101**,** 126-131.

Navarro-Llopis, V., Femenia, B., 2004. La utilización de semioquímicos en el control y seguimiento de las plagas agrícolas: aplicación sobre Cydia pomonella (Linnaeus) y Ceratitis capitata (Wiedemann). Phytoma España 164**,** 91-93.

Navarro-Llopis, V.N., Sanchis-Cabanes, J., Ayala, I., Casana-Giner, V., Primo-Yufera, E., 2004. Efficacy of lufenuron as chemosterilant against Ceratitis capitata in field trials. Pest Management Science 60**,** 914-920.

Ortu, S., Cocco, A., Dau, R., 2009. Evaluation of the entomopathogenic fungus Beauveria bassiana strain ATCC 74040 for the management of Ceratitis capitata. Bulletin of Insectology 62**,** 245-252.

Ovruski, S.M., Schliserman, P., Nunez-Campero, S.R., Orono, L.E., Bezdjian, L.P., Albornoz-Medina, P., Nieuwenhove, G.A.v., 2009. A survey of hymenopterous larval-pupal parasitoids associated with Anastrepha fraterculus and Ceratitis capitata (Diptera: Tephritidae) infesting wild guava (Psidium guajava) and peach (Prunus persica) in the southernmost section of the Bolivian Yungas forest. Florida Entomologist 92**,** 269-275.

Palou, L., Del Río, M.A., Jacas, J.A., 2007. Aplicación en poscosecha de cítricos de tratamientos cuarentenarios contra la mosca mediterránea de la fruta. Levante Agrícola 385**,** 192-199.

Papadopoulos, N.T., Carey, J.R., Katsoyannos, B.I., Kouloussis, N.A., 1996. Overwintering of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. Annals of the Entomological Society of America 89**,** 526-534.

Papadopoulos, N.T., Katsoyannos, B.I., 2003. Field parasitism of Ceratitis capitata larvae by Aganaspis daci in Chios, Greece. Biocontrol 48**,** 191-195.

Paranhos, B.J., Costa, M.d.L.Z., Ovruski, S.M., Alves, R.M., Blummer, L., Walder, J.M.M., 2008. Offspring in response to parental female densities in the fruit fly parasitoid Diachasmimorpha longicaudata (Hymenoptera: Braconidae: Opiinae). Florida Entomologist 91**,** 628-635.

Passino, G.S., Bazzoni, E., Moretti, M.D.L., Prota, R., 1999. Effects of essential oil formulations on Ceratitis capitata Wied. (Dipt., Tephritidae) adult flies. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 123**,** 145-149.

Peck, S.L., McQuate, G.T., 2000. Field tests of environmentally friendly malathion replacements to suppress wild Mediterranean fruit fly (Diptera : Tephritidae) populations. Journal of Economic Entomology 93**,** 280-289.

Piñero, J.C., Mau, R.F.L., Vargas, R.I., 2009. Managing Oriental Fruit Fly (Diptera: Tephritidae), With Spinosad-Based Protein Bait Sprays and Sanitation in Papaya Orchards in Hawaii. Journal of Economic Entomology 102**,** 1123-1132.

Putruele, G., Mouques, J., 2005. Mass trapping of Ceratitis capitata (Diptera: Tephritidae) in Argentina. Development of improved attractants and their integration into Fruit Fly SIT management programmes, Vienna 5-7 May 2005, pp. 121.

Rendon, P., Sivinski, J., Holler, T., Bloem, K., Lopez, M., Martinez, A., Aluja, M., 2006. The effects of sterile males and two braconid parasitoids, Fopius arisanus (Sonan) and Diachasmimorpha krausii (Fullaway) (Hymenoptera), on caged populations of Mediterranean fruit flies, Ceratitis capitata (Wied.) (Diptera : Tephritidae) at various sites in Guatemala. Biological Control 36**,** 224-231.

Ros, J.P., 1988. La Mosca Mediterranea de la fruta, Ceratitis capitata Wied. Biología y métodos de control. Hojas Divulgadoras, Ministerio de Agricultura, Pesca y Alimentacion, España 8**,** 28.

Ros, J.P., Castillo, E., Wong, E., Olivero, J., Rubio, J.R., Marquez, A.L., 2005a. Development of traps and killing agents to improve the mass trapping techinique against Ceratitis capitata Wied. and Bactrocera oleae Gmel. (Diptera: Teprhitidae). Development of improved attractants and their integration into Fruit Fly SIT management programmes, Vienna 5-7 May 2005, pp. 27.

Ros, J.P., Gomila, J., Reurer, M., Pons, P., Castillo, E., 2002. The use of mass-trapping against Medfly (Ceratitis capitata (Wied.)) in a sustainable agriculture system on Minorca Island, Spain. Proceedings of the 6th International Symposium on fruit flies of economic importance, Stellenbosch, South Africa, 6-10 May 2002, pp. 361-364.

Ros, J.P., Wong, E., Castro, V., Castillo, E., 1997. La Trimetilamina: un efectivo potenciador de los atrayentes putrescina y acetato amónico para capturar las hembras de la mosca mediterránea de la fruta Ceratitis capitata Wied. (Diptera: Teprhitidae). Boletín de Sanidad Vegetal, Plagas 23**,** 515-521.

Ros, J.P., Wong, E., Olivero, J., Rubio, J.R., Marquez, A.L., Castillo, E., Blas, P., 2005b. Desarrollo de atrayentes y mosqueros para su integración en los programas de trampeo masivo contra la mosca de la fruta (Ceratitis capitata Wied.) y la del olivo (Bactrocera oleae Gmel.). Boletín de Sanidad Vegetal, Plagas 31, 599-609.

Rossler, Y., Ravins, E., Gomes, P.J., 2000. Sterile insect technique (SIT) in the near east - a transboundary bridge for development and peace. Crop Protection 19, 733-738.

Rousse, P., Gourdon, F., Roubaud, M., Chiroleu, F., Quilici, S., 2009. Biotic and abiotic factors affecting the flight activity of Fopius arisanus, an egg-pupal parasitoid of Fruit Fly pests. Environmental Entomology 38.

Salgado, V.L., 1998. Studies on the mode of action of spinosad: Insect symptoms and physiological correlates. Pesticide Biochemistry and Physiology 60, 91-102.

Salvatore, A., Borkosky, S., Willink, E., Bardon, A., 2004. Toxic effects of lemon peel constituents on Ceratitis capitata. Journal of Chemical Ecology 30, 323-333.

San-Andrés, V., Beitia, F., Castañera, P., Sabater-Muñoz, B., 2009. Identificación de parasitoides de Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) mediante un sistema de PCR multiplex. VI Congreso Nacional de Entomología Aplicada, Palma de Mallorca 19-23 Octubre, 2009, pp. 55.

Sapountzis, P., Moraiti, K., Kounatidis, I., Siozios, S., Mavragani-Tsipidou, P., Papadopoulos, N., Bourtzis, K., 2008. Wolbachia symbiosis in Tephritidae. First Meeting of TEAM, Palma de Mallorca 7-8 April, 2008, pp. 13.

Sarasua, M.J., Santiago-Álvarez, C., 1983. Effect of diflubenzuron on the fecundity of Ceratitis capitata. Entomologia Experimentalis Et Applicata 33, 223-225.

Sastre, C., Melo, J.C., Borreli, G., 1999. La captura de hembras: una posible salida en el control de mosca de la fruta (Ceratitis capitata, Wied.) en melocotonero. Phytoma España 113, 42-47.

Saul, S.H., Tsuda, D., Wong, T.T.Y., 1983. Laboratory and field trials of soil applications of Methoprene and other insecticides for control of the Mediterranean fruit-fly (Diptera: Tephritidae). Journal of Economic Entomology 76, 174-177.

Schetelig, M.F., Caceres, C., Zacharopoulou, A., Franz, G., Wimmer, E.A., 2009. Conditional embryonic lethality to improve the sterile insect technique in Ceratitis capitata (Diptera: Tephritidae). BMC Biol 7, 4.

Sessler, B., Wiegand, G., Becker, T., 2004. Integrated Fruit Production systems in Europe: comparing guidelines and organisations in Spain, Italy, the Netherlands and France. 6th International Conference on Integrated Fruit Production (IOBC-OILB), Baselga di Piné, 26-30 September 2004, pp. 29.

Shoukry, A., Hafez, M., 1979. Studies on the biology of the Mediterranean fruit fly Ceratitis capitata. Entomologia Experimentalis Et Applicata 26, 33-39.

Siskos, E.P., Konstantopoulou, M.A., Mazomenos, B.E., 2009. Insecticidal activity of Citrus aurantium peel extract against Bactrocera oleae and Ceratitis capitata adults (Diptera: Tephritidae). Journal of Applied Entomology 133, 108-116.

Sivinski, J., Jeronimo, F., Holler, T., 2000. Development of aerial releases of Diachasmimorpha tryoni (Cameron) (Hymenoptera : Braconidae), a parasitoid that attacks the Mediterranean fruit fly, Ceratitis capitata

(Weidemann) (Diptera : Tephritidae), in the Guatemalan highlands. Biocontrol Science and Technology 10, 15-25.

Stark, J.D., Vargas, R., Miller, N., 2004. Toxicity of spinosad in protein bait to three economically important tephritid fruit fly species (Diptera : Tephritidae) and their parasitoids (Hymenoptera : Braconidae). Journal of Economic Entomology 97, 911-915.

Steiner, L.F., 1952. Fruit fly control in Hawaii with poison-bait sprays containing protein hydrolisates. Journal of Economic Entomology 45, 838-843.

Stern, V.M., Smith, R.F., Van den Bosch, R., And Hagen, K.S., 1959. The integrated control concept. Hilgardia 29, 81-101.

Torné, M., 2008. Ceratitis capitata: situación actual y perspectivas de futuro. Jornada tècnica: problemàtica actual en el control dels paràsits dels fruiters. Perspectives de futur, Lleida 26 Setembre, 2008, pp. 14.

Torres-Rivera, Z., Hallman, G.J., 2007. Low dose irradiation phytosanitary treatment against Mediterranean fruit fly (Diptera : Tephritidae). Florida Entomologist 90, 343-346.

Torà, R., 2008. Mètodes biotècnics: la confusió sexual i la captura massiva. Perspectives de futur. Jornada Tècnica: Problemàtica actual en el control dels paràsits dels fruiters. Perspectives de futur, Lleida 26 Setembre, 2008, pp. 46.

Urbaneja, A., Mari, F.G., Tortosa, D., Navarro, C., Vanaclocha, P., Bargues, L., Castanera, P., 2006. Influence of ground predators on the survival of the mediterranean fruit fly pupae, Ceratitis capitata, in Spanish citrus orchards. Biocontrol 51, 611-626.

Vayssieres, J.F., Sinzogan, A., Korie, S., I., O., A., T.-O., 2009. Effectiveness of Spinosad Bait sprays (GF-120) in controlling mango-infesting Fruit Flies (Diptera: Tephritidae) in Benin. Journal of Economic Entomology 102, 515-521.

Vergoulas, P., Torné, M., 2003. Spinosad cebo: un sistema innovador para el control de la mosca del mediterráneo. Phytoma España 153, 134-138.

Vidal, J.C., Aboussaid, H., Oufdou, K., El Messoussi, S., Castañera, P., González-Cabrera, J., 2008. Characterization of Bacillus thuringiensis strain collections from Spain and Morocco ecosystems and evaluation of their insecticidal activity against Ceratitis capitata (Diptera: Tephritidae). First Meeting of TEAM, Palma de Mallorca 7-8 April, 2008, pp. 41.

Vidal, J.C., Castañera, P., González, J., 2009. Estrategias para la utilización de la tecnología Bt en el control de Ceratitis capitata (Wiedemann) (Diptera: Tephritidae). VI Congreso Nacional de Entomología Aplicada, Palma de Mallorca 19-23 Octubre, 2009, pp. 51.

Viscarret, M.M., La Rossa, R., Segura, D.F., Ovruski, S.M., Cladera, J.L., 2006. Evaluation of the parasitoid Diachasmimorpha longicaudata (Ashmead) (Hymenoptera : Braconidae) reared on a genetic sexing strain of Ceratitis capitata (Wied.) (Diptera : Tephritidae). Biological Control 36, 147-153.

Vogt, H., 2004. Results from a field study to monitor effects of Spinosad on the natural community in an apple orchard. 6th International Conference on Integrated Fruit Production (IOBC-OILB), Baselga di Piné, 26-30 September 2004, pp. 111.

Wang, X.G., Messing, R.H., 2003. Intra- and interspecific competition by Fopius arisanus and Diachasmimorpha tryoni (Hymenoptera : Braconidae), parasitoids of tephritid fruit flies. Biological Control 27, 251-259.

Weems, H.V., Jr., 1981. Mediterranean fruit fly, Ceratitis capitata (Wiedemann) (Diptera: Tephritidae). Entomology Circular, Division of Plant Industry, Florida Department of Agriculture and Consumer Services**,** 12 pp.

White, I.M., Elson-Harris, M.M., 1992. Fruit flies of economic significance: Their identification and bionomics. Wallingford, Oxon, CAB International.

Zabalou, S., Apostolaki, A., Livadaras, I., Franz, G., Robinson, A.S., Savakis, C., Bourtzis, K., 2009. Incompatible insect technique: incompatible males from a Ceratitis capitata genetic sexing strain. Entomologia Experimentalis Et Applicata 132**,** 232-240.

Zapata, N., Budia, F., Vinuela, E., Medina, P., 2006. Insecticidal effects of various concentrations of selected extractions of Cestrum parqui on adult and immature Ceratitis capitata. Journal of Economic Entomology 99**,** 359-365.

Zou, X.B., Zhao, J.W., Li, Y.X., Holmes, M., 2010. In-line detection of apple defects using three color cameras system. Computers and Electronics in Agriculture 70**,** 129-134.

2. GENERAL OBJECTIVES

GENERAL OBJECTIVES

Over the last years, overwintering studies have been carried out in the Mediterranean basin and the efficacy of the mass trapping technique in fruit orchards against medfly has been confirmed by researchers in several countries. The general aim of this Ph.D. is to increase the knowledge on these two fields in order to control the pest in two different areas where it is established.

The specific aims of the present work are:

1. To estimate the survival of three stages of medfly in the North-East extreme of Spain under natural winter conditions:
    a. To determine the survival of wild larva and pupa through the collection of infested fruits.
    b. To find out the survival of pupa stage in a commercial apple orchard, over two crop seasons.
    c. To find out the survival of adult stage in a commercial apple orchard, over two crop seasons.

2. To evaluate in the field the effectiveness of trapping equipment for *Ceratitis* spp. in La Réunion Island through comparative studies of:
    a. Traps.
    b. Attractants.
    c. Insecticides.
    d. Systems (commercial complete equipments).

3. To evaluate different characteristics of the insecticide to be used in mass trapping technique against medfly:
    a. To find an active ingredient and formulation at least as effective as DDVP.
    b. To identify the optimal location of the insecticide in the trap.
    c. To ascertain the efficacy of the prototype carrying the selected insecticide.

4. To improve the efficacy of mass trapping technique in Girona area:
    a. To describe the pest colonization process at orchard level in peach orchards.
    b. To test the level of fruit protection in peach orchards where mass trapping methodology is used.
    c. To determine the minimum proportion of traps that must be checked to ensure a reliable estimate of the captured population.

3. CHAPTER I: Survival of wild larvae and overwinter of pupa and adult stages of *Ceratitis capitata* under natural winter conditions of the Girona area

INDEX

1 INTRODUCTION 59

2 MATERIAL AND METHODS 61

2.1 OVERWINTERING OF LARVAE AND PUPAE IN NATURALLY INFESTED APPLES 61

2.2 PUPAE TRIAL 63

2.3 ADULTS TRIAL 65

3 RESULTS 67

3.1 OVERWINTERING OF LARVAE AND PUPAE IN NATURALLY INFESTED APPLES 67

3.2 PUPAE TRIAL 69

3.3 ADULTS TRIAL 72

4 DISCUSSION 78

4.1 OVERWINTERING OF LARVAE AND PUPAE IN NATURALLY INFESTED APPLES 78

4.2 PUPAE TRIAL 82

4.3 ADULTS TRIAL 85

5 CONCLUSIONS 88

6 REFERENCES 88

1 INTRODUCTION

Ceratitis capitata (Wiedemann) is well adapted to different crops, different host plants and to the overall climatic conditions from each area. At a given time and place, its population exists at all stages of its development, in different environments: eggs (fruits), larvae (fruits and soil in the case of mature larvae), pupae (soil) and adults (tree canopy) (Carey, 1984). The present study was carried out on two of these environments: fruits and soil, in order to improve detailed knowledge of the overwintering of this species in the Girona area.

In some areas of the Mediterranean basin, medfly population density is controlled at least once a year by temperature (Miranda et al., 2001). Therefore, knowledge is required of the strategy which this insect uses to survive the most hostile periods during winter in order to design effective control methodologies to fight against it.

Different stages of medfly overwinter in different ways in the areas where it has become established. Adults of this species are present throughout the year in several areas, including the Southern coast of Spain (Fimiani, 1989) and the Eastern coast, particularly in Tarragona and Valencia (Martínez-Ferrer et al., 2007). In these two areas, the availability of ripe fruit facilitates their flight throughout winter, albeit in very low levels from February to April (Martínez-Ferrer et al., 2007). In Valencia, eggs laid in October develop into larvae and pupae from November to December and eventually emerge as adults in February and March, the survivors producing their first offspring of the year (De Andrés and García-Marí, 2007). Medfly adults have not been recorded over the coldest period of the year in the Girona area (Escudero-Colomar et al., 2008).

There are two overwintering hypotheses regarding the appearance of new populations after winter season. The first assumes that infestations are of a temporary nature originating from infested fruit imported from warmer areas which contain a large quantity of winter hosts (Romani, 1997) (Israely et al., 2004). This theory had been argued in a number of areas including the central mountains of Israel (Israely et al., 2004) (Israely et al., 2005), the Balearic Islands (Miranda et al., 2001) and Southern France (Cayol and Causse, 1993). The second theory assumes that *C. capitata* adapts to temperate weather because it is resistant to low temperatures (Carey, 1991) (Romani, 1997) and that this enhances successful overwintering of part of the population, as seen in Central Italy (Sciarretta et al., 2009). In Northern Italy, adults have been proved to overwinter in sheltered parts of indoor environments (Rigamonti et al., 2002) (Rigamonti, 2004b). In Northern Greece, medfly has been observed to overwinter as larvae inside fruits even below zero temperature (Papadopoulos et al., 1996), and in Tarragona they were found inside late-ripening varieties of orange (Martínez-Ferrer et al., 2006), although further studies must be done to corroborate this evidence.

Survival of a small percentage of individuals each winter and regeneration of the entire population from these individuals in spring and early summer probably constitutes a strong selection pressure in this insect for the development of a mechanism to withstand the cold (Papadopoulos et al., 1996).

Studies of the population dynamics of medfly have shown that the main factor affecting population build-up in the tropics is the abundance and availability of fruit, whereas in temperate areas such as Northern Greece, low winter temperatures and the absence of host fruits for a long period are the two main factors that inhibit overwintering (Israely et al., 1997) (Papadopoulos et al., 2001a) (Papadopoulos et al., 2001b). Temperature can also affect the appearance of the population after winter period, retarding or advancing the presence of individuals, as seen in Girona (Escudero-Colomar et al., 2008) and Italy (Trematerra et al., 2008). Although distribution of the insect appears to be ultimately restricted by the severity of the winter, the existence of a variety of micro-climates in a particular area means that other climatic factors may limit or at least modulate the population dynamics of the species (Vera et al., 2002). In a study of the geographical distribution of medfly in different areas using the program CLIMEX, the main limiting factor to survive the winter was found to be cold stress. The program did not exclude the lethal effect of low temperature but extreme temperatures appeared to be less restrictive to the distribution than the limitation imposed by the need for thermal accumulation in winter (Vera et al., 2002). The pupa stage is the most sensitive to temperature, which directly affects its survival or indirectly diminishes the activity of adults due to the thermal stress suffered in their previous stage (Segura et al., 2004). Minimum air and subsoil temperatures are therefore important factors to be taken into account in the study of the medfly survival. Nevertheless, survival in insects depends on both, the temperature and the duration of exposure (Denlinger and Lee, 1998) and one of the measures of the medfly's cold tolerance is the duration of low temperatures (Turnock and Fields, 2005). Therefore, the combination of factors such as dry and cold stress (Vera et al., 2002) and duration of low temperatures could be responsible for the low incidence of the pest in a particular location.

In Greece, apples are known to be host fruits that last through the winter in a good enough condition to provide a suitable refuge for larvae, to favor slow growth and to provide protection from natural enemies especially in conjunction with low winter temperatures, enabling the larval stage to survive through the winter (Papadopoulos et al., 1996). Therefore, this was the crop chosen in the present study.

Although recent studies performed in Girona support the hypothesis of an overwintering population in this area (Escudero-Colomar et al., 2008) the conditions of overwintering of the different stages of medfly development in the North-East extreme of Spain are still unknown. In order to improve understanding of this issue, a number of objectives were considered.

The aim of this study was to estimate the survival rates of the larvae, pupae and adult stages of medfly in the North-East extreme of Spain under natural winter conditions. Three experiments were designed for this purpose.

The aim of the first experiment was to determine the survival rate of wild larvae and pupae through the collection of infested apples. The aim of the reminder experiments was to ascertain the survival rates of pupa and adult stages in a commercial apple orchard, over a period of two years (two winter seasons).

2 MATERIAL AND METHODS

2.1 OVERWINTERING OF LARVAE AND PUPAE IN NATURALLY INFESTED APPLES

The trial took place between October 2007 and March 2008.

Three hundred infested apples were picked from a commercial orchard located in a Girona fruit growing area. Half were ‘Golden Delicious’ and the remainder were of the ‘Granny Smith’ cultivar. ‘Golden Delicious’ apples were collected on 1st and 2nd, October and ‘Granny Smith’ on 22nd and 23rd November. Fruits of each variety were divided into two groups (replicates), each of 50 apples, which were weighed and held in a 3 m high plastic tunnel without lateral walls, in order to simulate natural winter conditions while being sheltered from the rain. The other two replicates of 25 fruits were placed in a chamber in which conditions were controlled (25ºC ± 2ºC and 50 - 80% R.H.).

Apples were placed on raised metallic grids in plastic trays with water at the bottom, and covered by a tulle cloth to avoid possible damage from ants or other insects. Due to the extreme temperatures in mid November, the water froze and had to be changed to cellulose absorbent paper.

The trays from outdoors were covered with evolutionary cages (60 x 60 x 60 cm) specially designed to support external environmental conditions, in order to avoid the effect of birds and pollution of the rotten fruits.

To avoid possible displacement of the material by the high winds that often occur in the survey area of Girona, all evolutionary cages situated outside were fixed to a wooden support, 70 cm above the ground, using elastic ropes (Figures 4 and 5).

Figures 4 and 5. Naturally infested apples placed in external conditions and covered by the evolutionary cages. Cages placed under the plastic tunnel. Photos: E. Peñarrubia.

The methodology consisted of a daily collection of third-instar larvae that had jumped from fruits to the water, using soft metal tweezers. The larvae collected were placed in plexiglas petri dishes without substrate, labelled (with date, replicate and apple variety) and installed in other evolutionary cages also under outdoor conditions.

Development to pupa and adult stages was monitored daily from Monday to Friday (not at weekends), and the emerging individuals were sexed. The survival rate for medfly larvae after two days submerged in water is 44% and zero after four days of submersion (Eskafi and Fernández, 1990), larvae collected on Monday morning were therefore counted but eliminated from the study because they could have been submerged in water from Friday and a high percentage could have drowned.

When no registration of larvae coming from fruits was checked for up to fifteen days the monitoring of fruits was considered finished.

Over the experimental period, meteorological data were registered in a weather station located 50 m from the survey area (DAR, 2008). Temperature and relative humidity (R.H.) from inside the evolutionary cages and outdoors were recorded hourly using data loggers (Hobo® Pro V2 - ext. Temp/RH, Onset Company). The same model of data logger was used inside the chamber in order to verify the range of temperature and relative humidity.

The variables measured in this winter trial were: the number of days from placing the apples to the emergence of mature larvae; the number of days from placing the fruit to pupation of the medfly; the mortality rate of larvae once they have left the fruit; the duration of pupal development; the pupae mortality rate; the adult emergence and the sex ratio of the emerged adults.

From this data were calculated the average time from the beginning of the trial until the exit of larvae, the length of the mature larva stage from the emergence of fruit until pupal instar, of the pupal development and the average percentages of mature larval mortality, adult emergence and each gender.

2.2 PUPAE TRIAL

This trial was performed over two consecutive winters: 2008 - 2009 and 2009 - 2010, terminating in August 2009 and August 2010, respectively.

The study was performed in a 962 m^2 commercial plot of 'Golden Delicious' apples, oriented towards the North, enclosed by a wood structure with walls and roof made of plastic mesh. The plot was divided into three sealed compartments with the same dimensions, each with its own independent access door (Figure 6). Climatic conditions including temperature and relative humidity could differ among the three compartments.

Figure 6. Closed commercial plot where the pupae and adult experiments took place. Photo: E. Peñarrubia.

As in the previous trial, the control treatment took place in a chamber with controlled conditions (25ºC ± 1ºC, 50 - 80% R.H. and in completely darkness), with the aim of verifying the viability of the population used in the field treatment.

The experiment was carried out on a sandy-loam soil surface with a high sand content, which had a very low water retention capacity. Three hundred and sixty pupae of one to two days old from a F1 autochthonous population that had been reared under controlled conditions in Girona were buried. They were buried in groups of 10 individuals inside plastic glasses 2 cm below the surface with 5 cm of soil below them. In the second year, because soil compaction had been found in the previous trial, soil used in the glasses was mixed with a small proportion of perlite, a spherical mineral that has relatively high water content, is porous and light enhancing. This improved the quality of the substrate, by providing greater aeration and increasing the retention of superficial water.

The glasses used were of one litre capacity and open at the bottom. A plastic mesh 1 x 1 mm was attached to the top, in order to prevent the entrance of predators but allowing the percolation of rainy water. The open top of the glasses was also covered with plastic mesh held in place by elastic bands to facilitate the entrance of humidity and thus simulate the external conditions.

Glasses were buried 5 cm deep in the centre of corridors between rows of trees and were labelled with the date and position (Figure 7).

Figure 7. Plastic glass containing ten pupae and soil mixed with perlite. Behind the glass is the data logger which measured the temperature of the subsoil. Photo: E. Peñarrubia.

The trial was carried out three times over the study period, each of nine replicates in the space, distributed by three glasses per compartment. In the chamber under controlled conditions, three glasses were used in each time that the trial was carried out, as the control. These glasses were filled with sand from the soil corresponding to each of the three compartments of the plot. Overall, 270 pupae were maintained under natural winter conditions and 90 more were maintained under controlled ones.

Once the glasses were distributed in the plot or inside the chamber, the emergence of adults was checked three times per week throughout the first months of trial, but from the first appearance of an adult medfly in the Girona fruit growing area (27th May, 2009 and 7th June, 2010) until the end of the study, checks were carried out daily.

The trial ended on 1st July of both years, corresponding to 35 and 24 days after the detection of the first capture of adult medfly in Girona province. This detection was carried out using a monitoring net of 50 traps placed in the Girona fruit growing area with an average of one trap per orchard, which was checked weekly. After the removal of the glasses, all were checked for undeveloped pupae and the presence of individuals of other species which could have acted as predators. Other insects found inside the glasses were collected and identified.

Over both experimental periods, meteorological data were registered in a weather station located 700 m from the survey plot (DAR, 2010). Temperature and relative humidity inside each of the compartments of the plot and in the chamber were registered hourly by a data logger hung 1.60 m above ground level (Hobo® Pro V2 - ext. Temp/RH, Onset Company). One data logger was placed in each compartment. In the second year a special temperature sensor was located inside the closed plot (Hobo® Pro V2 - Temp/ext. temp, Onset

Company), buried 5 cm, in order to register the temperature where the pupae had been placed.

The threshold of pupae development for medfly was thought to be 11.2ºC (Duyck and Quilici, 2002) and in this experiment, meteorological data was used to calculate the number of hours during which the temperature was below this threshold, named 'cold hours below 11.2ºC' (DAR, 2010). Unfortunately, it was not possible to specify the temperature accurately in units of less than 1º, so the threshold used was 11ºC.

The variables measured in this trial were: duration of pupal development; pupae mortality; pupae recovered at the end of the trial; pupae dried up over the study period; insects from other species and climatic conditions (air temperature in the area and inside the compartments, soil temperature at 5 cm, relative humidity in the compartments and rainfall).

From this information it was calculated the average time span for pupa development, the percentage of pupae surviving, recovered and dried up and the number of hours when the temperature was below 11ºC in outdoor conditions and at 5 cm below the surface.

2.3 ADULTS TRIAL

This trial was performed over two consecutive winters: between mid December 2008 and mid January 2009 and from mid November 2009 to late December 2009.

The study took place in the same plot as that used for the pupae trial described above. The control treatment was arranged in a chamber maintained at 25ºC ± 1ºC, 50 - 80% R.H. with a photoperiod of 14 hours of light and 10 hours of darkness, in order to verify the viability and longevity of the population used in the field.

In 2008, 236 five to seven day old second generation (F2) adults were used and in the following year, 212 six day old wild flies were used, originally from an autochthonous population reared under controlled conditions in Girona.

Individuals were placed in evolutionary cages provided with water and solid food diet ad libitum, composed respectively of 4:1:5 parts of hydrolysed protein, sugar and water. In the second year, a mesh was placed in the base of the cages in order to avoid the possible sinking of flies into rain water. Each evolutionary cage was placed on two wooden supports in order to avoid direct contact with the ground. They were also fixed to the soil surface with two iron pegs, 1.5 cm of diameter, to avoid movement by the wind (Figures 8 and 9).

Figures 8 and 9. Evolutionary cage with food, water and a data logger. General view of one cage inside the closed plot. Photos: E. Peñarrubia.

In three replicates, 45 to 60 individuals were placed in each cage on 16th December, 2008 and 16th November, 2009. The evolutionary cages were evenly distributed among the compartments of the plot and were maintained under natural winter conditions, while the control cage was managed under chamber conditions. Once the cages had been installed, individual deaths were checked daily until the demise of the last adult.

Over both experimental periods, meteorological data were recorded in a weather station located 700 m from the survey plot (DAR, 2010). Temperature and relative humidity in the compartments of the plot and in the chamber were registered hourly using data loggers hung 1.60 m above ground level (Hobo® Pro V2 - ext. Temp/RH, Onset Company).

The lower developmental threshold for ovarian maturation (8.9ºC) is a stricter data than the lower threshold for population growth (Duyck and Quilici, 2002) and was therefore used to calculate cold hours in the adult experiment (DAR, 2010). As mentioned in the previous trial, it was not possible to calculate units smaller than 1ºC, so the threshold used to calculate cold hours in this experiment was 9ºC.

Variables measured in this trial were: daily adult mortality; climatic conditions including hourly air temperature and relative humidity inside the evolutionary cages and outside the plot, rainfall, speed and direction of the wind.

From this information, the mean and maximum number of days which adults survived was calculated. The survival analysis was carried out using the Kaplan-Meier methodology, i.e. by obtaining estimates of the mean survival and their standard error. A comparison of the survival curves was carried out using the statistic of contrast for equality of survival distributions (Chi-squared with significance level of 0.05, used with the prove Log-rank), and comparing the different treatments two by two. Thus, the contrast of hypothesis was:

H_0: both distributions of survival are equal.

H_1: the two distributions of survival are different.

This statistical analysis was carried out using the software SPSS v.15.

Calculations were made of the percentage of survivors related to maximum and minimum temperatures, the age of flies when mortality of individuals reached 50% and the number of cold hours below 9ºC.

It was studied the relation between the factors: survival percentage of flies, age of adults, daily minimum temperature, daily maximum temperature, daily average temperature and daily rainfall, using the factor analysis to obtain the Kaiser's measure of sampling adequacy (MSA) and using the principal components analysis (PCA) through the software Enterprise guide v. 4.2 of SAS program.

3 RESULTS

3.1 OVERWINTERING OF LARVAE AND PUPAE IN NATURALLY INFESTED APPLES

A total number of 2,039 larvae were found in 'Golden Delicious' apples, and 26 in 'Granny Smith'. From these values 657 larvae from 'Golden Delicious' variety were evaluated under natural conditions and just one from 'Granny Smith'. The total number of larvae evaluated from indoors were 521, being 504 and 17 from each variety, respectively. Of all larvae registered, 65.84 larvae/kg were found in 'Golden Delicious' apples maintained outdoors and 136.96 larvae/kg in fruits in chamber. In 'Granny Smith' apples, only 0.08 and 3.25 larvae/kg were recorded in outdoor and indoor conditions respectively.

The average time and the standard error from the beginning of the experiment until the mature larvae left the 'Golden Delicious' fruits were 22.73 ± 0.49 days in exterior conditions and 13.95 ± 0.2 days in controlled ones. For the 'Granny Smith' variety in chamber conditions this value was 14.59 ± 1.3 days. The maximum number of days of larval development until their exit from fruits was 79 days in exterior conditions and 29 days in the chamber. The last third stage larvae from 'Golden Delicious' outdoor fruits was collected on December 20th, while under controlled conditions the last larvae was recorded on October 30th. The last mature larva from the 'Granny Smith' variety was found on December 18th.

In outdoors, the average length of the mature larva stage from the emergence from 'Golden Delicious' apples until pupal instar was 2.51 ± 0.07 days. In controlled circumstances this value was 3.32 ± 0.12 days for larvae emerging from the 'Golden Delicious' variety and 2.29 ± 0.32 days for larvae found in 'Granny Smith'. The average larvae mortality rate registered in 'Golden

Delicious' was 3.16% for those outdoors and 4.67% for those under chamber conditions. In the late variety, inside the chamber the value was null.

In outdoors, average pupal development time for individuals emerging from 'Golden Delicious' fruits was 29.35 ± 0.69 days. In chamber conditions this value was 11.63 ± 0.14 days for pupae from 'Golden Delicious' apples and 12.08 ± 0.43 days for pupae from the 'Granny Smith' variety. The first adults emerged during the last week of October after an average of 22 days of pupal development time, while adults emerging in mid January took 57 days (Figure 10). There appeared to be a big difference between the average lengths of the pupa stage of each replicate in respect of 'Golden Delicious' fruits. However, in the first replicate from 5th of December to the end of the trial, only five individuals were recorded and in the other replicate a single adult was observed, which died while emerging from its pupae.

Pupae mortality in the primary variety was 78.90% outdoors and 38.71% in controlled conditions. The only pupae obtained from 'Granny Smith' apples under natural conditions died during this stage and in the chamber, pupal mortality was 28.57%.

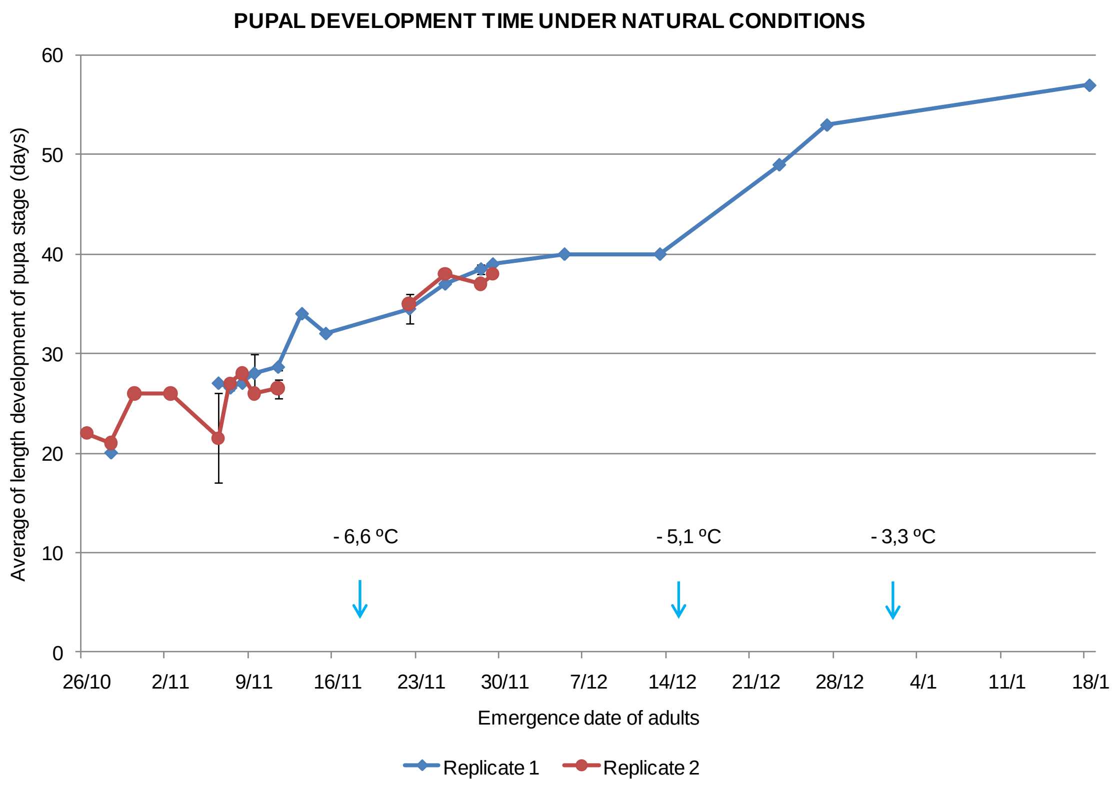


Figure 10. Average and standard error of development time of pupa stage for individuals emerging from 'Golden Delicious' fruits under natural winter conditions over the time. Arrows show the monthly minimal absolute temperature registered in the surveyed area.

Over the period of this trial the generalist parasite or hyperparasite *Melittobia acasta* (Walker) was found on medfly pupae (identified by Dr. María Jesús Verdú, IVIA). Owing to the traceability of this experiment, it is known that the egg of this parasite was laid on the medfly larvae while the fruit was still on the commercial plot.

The average percentage of adult emergence from ‘Golden Delicious’ apples at field level was 14.15%, corresponding to 5.76 adults/kg. Under controlled conditions for the same variety, the average rate of adult emergence was 51.73%, equivalent to 34.85 adults/kg, while for ‘Granny Smith’ it was 71.43%, corresponding to 1.59 adults/kg. The last emergence of adults from ‘Golden Delicious’ in natural conditions fruits took place on January 18th and inside the chamber, on November 12th. The last adult emerging from the later variety in indoor conditions was observed on December the 30th.

The percentage of each gender emerging from ‘Golden Delicious’ fruits maintained in external conditions was 48.42% male and 51.58% female and for those reared inside the chamber: 49.10% male and 50.90% female.

The average monthly minimum outdoor temperature registered for each month from October to February was 10ºC, 3.2ºC, 0.5ºC, 2.5ºC and 2.9ºC, respectively, and the absolute minimum air temperature in these same moths was 2.3ºC, - 6.6ºC, - 5.1ºC, - 3.3ºC and - 1.6ºC. The average relative humidity over the study period registered in the outdoor cages was 72.15%, with a minimum of 18.86% and a maximum of 94.48%.

3.2 PUPAE TRIAL

The average length of the pupa stage and its standard error on each of the three occasions that the trial was conducted during the study was 10.53 ± 0.52 days, 11.73 ± 0.27 days and 11.08 ± 0.08 days in the first winter and in the second winter 11 ± 0 days, 11 ± 0 days and 10 ± 0 days.

After the eight months of study of each year, pupal survival under natural conditions was null. In the chamber, however, average adult emergence rates for each of the three periods studied in the years 2008 - 2009 were 56.67%, 50 and 80%. For the years 2009 - 2010 they were 76.67, 33.33 and 63.33%.

At the end of the trial, all outdoor glasses were checked and 39.63 and 40% of pupae were found each year. However, in controlled conditions, the percentages of pupae found at the end of the trial period were 98.90 and 96.70%. Some of the pupae found were dried up (3 and 6.30% in outdoors and 5.56 and 2.22% in indoors for each year, respectively). A small number of these pupae had holes in the middle of the cocoon, and the interior was completely empty. From the twenty one insects recorded inside the pots under natural conditions four of them were potential predators of pupae. All specimens were ants from the species *Formica cunicularia* (Latreille). Two of them were found during the first winter season in the Northern part of the plot and the other two in the second year, one in the Northern compartment and the other in the Southern part (Figure 11). All four specimens were placed in the collection of Dr. Silvia Abril at the University of Girona.

Figure 11. Pupae of *C. capitata* and individuals of *F. cunicularia* found at the end of the trial from 2009 - 2010. Photo: E. Peñarrubia.

Temperatures registered inside all three compartments on the plot were very similar, with maximum differences of ±1ºC. The minimum absolute temperature registered over the study period in the first winter was - 4.4ºC under natural conditions and 3.7ºC at 5 cm below surface (Figure 12). In the second winter, the minimum absolute temperature on the surface was - 8.3ºC and 1.5ºC in the subsoil. The subsoil temperature was maintained in the threshold between the maximum and minimum daily temperatures over both years of study (Figure 12).

Analysis of climatic data showed that, at five centimeters below the surface, there had been some periods of low temperature followed by several days without rainfall. During the first winter season, these periods occurred on 12th December, 2009, when the minimum temperature in the soil fell to 4.2ºC, and on 16th February, 2009, when it dropped to 4.3ºC, while in the second year, on 28th January, 2010, the minimum subsoil temperature was 5.4ºC.

The number of cold hours below the threshold of pupae development (11ºC) in the first winter season was 3,113 h (equivalent to 130 days or 4.32 months) and in the second, it was 2,893 h (equivalent to 120 days or 4 months) (Figure 13).

The number of cold hours below the threshold of pupae development (11ºC) at 5 cm below surface was 2,835 h (equivalent to 118 days or 3.94 months) in the first winter season and 2,235 h (equivalent to 93 days or 3.1 months) in the second (Figure 14).

Rainfall over the survey periods was 411 mm and 525 mm (Figure 12) for the eight months of trial in each year. However, extremely high levels were registered in a single day, or over a short period: 54.6 mm were recorded on 26th December, 2008; 30 mm on 3rd February, 2009; and 45.6 mm on 4th May,

2010. On 8th March, 2010 an atypical snowfall occurred in the area, leaving 55.1 mm of snow. Although thick snow accumulated on top of the field cage where the trial was performed, glasses containing pupae were protected by the mesh.

Relative humidity, recorded hourly inside each of the three compartments on the study plot was very similar, with an average of 75.71%, a minimum of 24.06% and a maximum of 95.05% over the first winter season, between 11th November, 2008 and 1st July, 2009. In the second year, between 12th November, 2009 and 1st July, 2010, the average, minimum and maximum relative humidity readings were 77.97%, 21.64 and 99.30%, respectively. In the first period, R.H. fell below 30% for only nine hours and in the second period for no more than six.

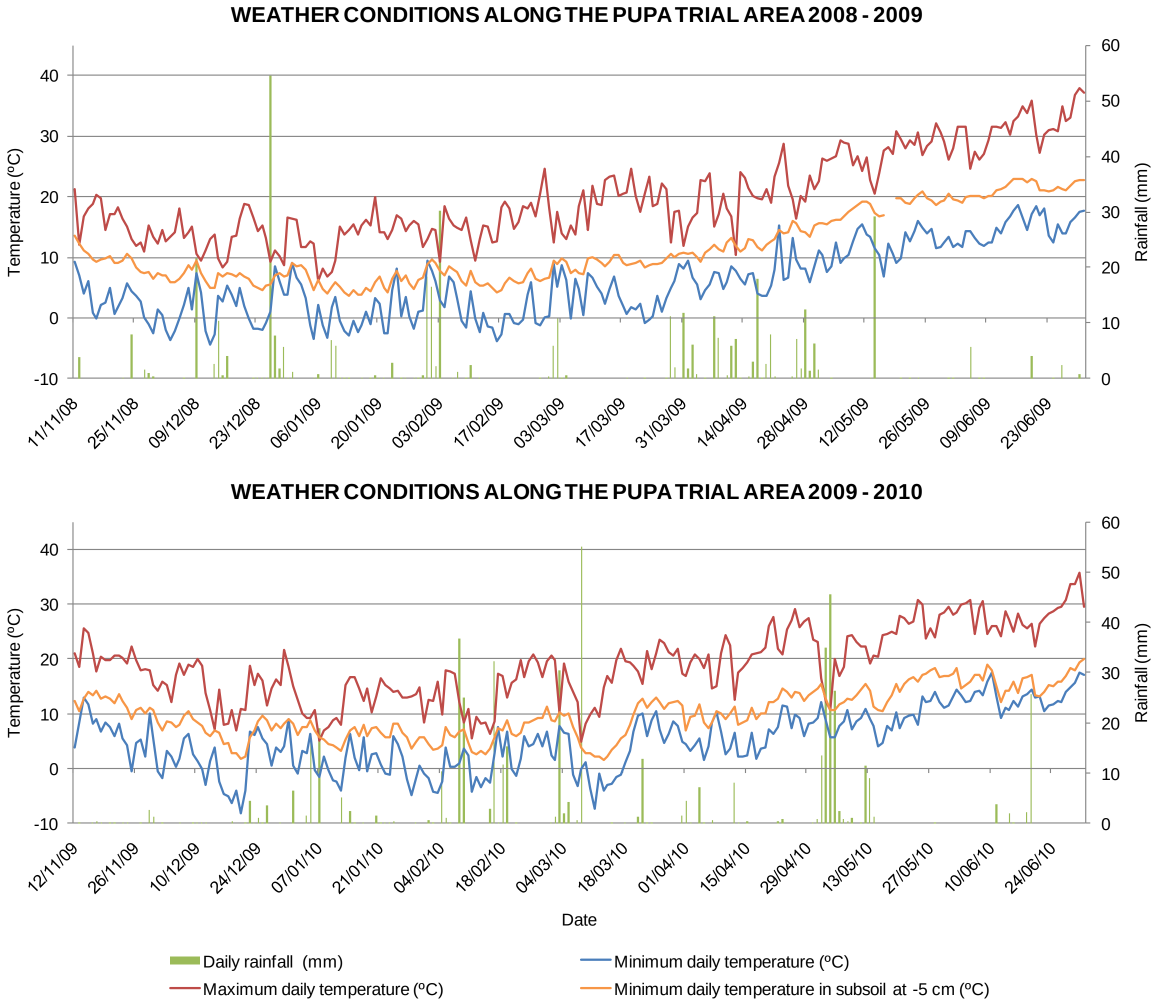


Figure 12. Climatic conditions registered over the two study periods: daily rainfall, minimum and maximum daily air temperature and minimum daily temperature at 5 cm below ground.

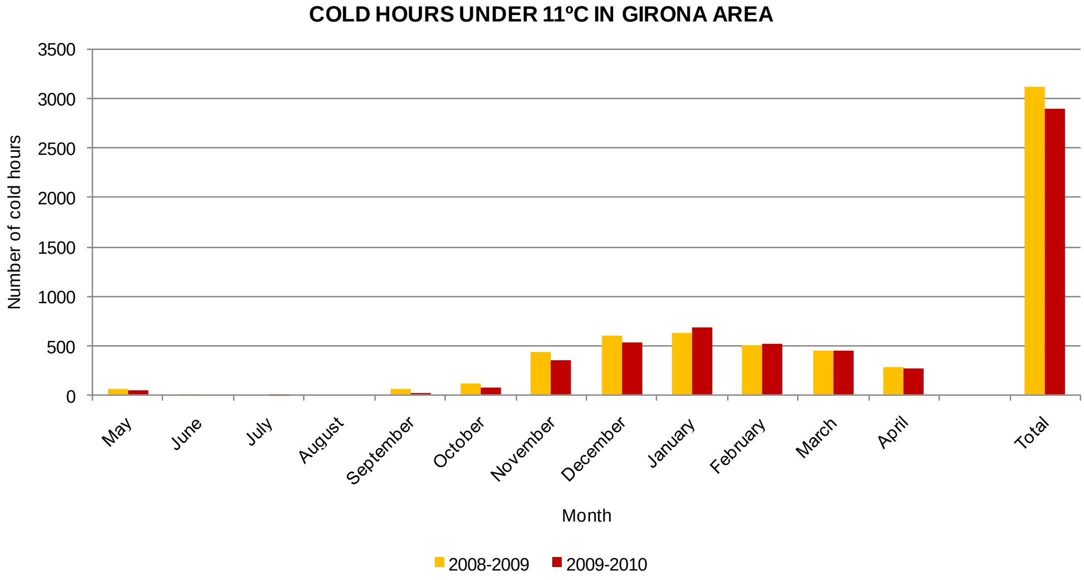


Figure 13. Number of hours registered under the development threshold of pupa, 11ºC at surface level in the two years studied.

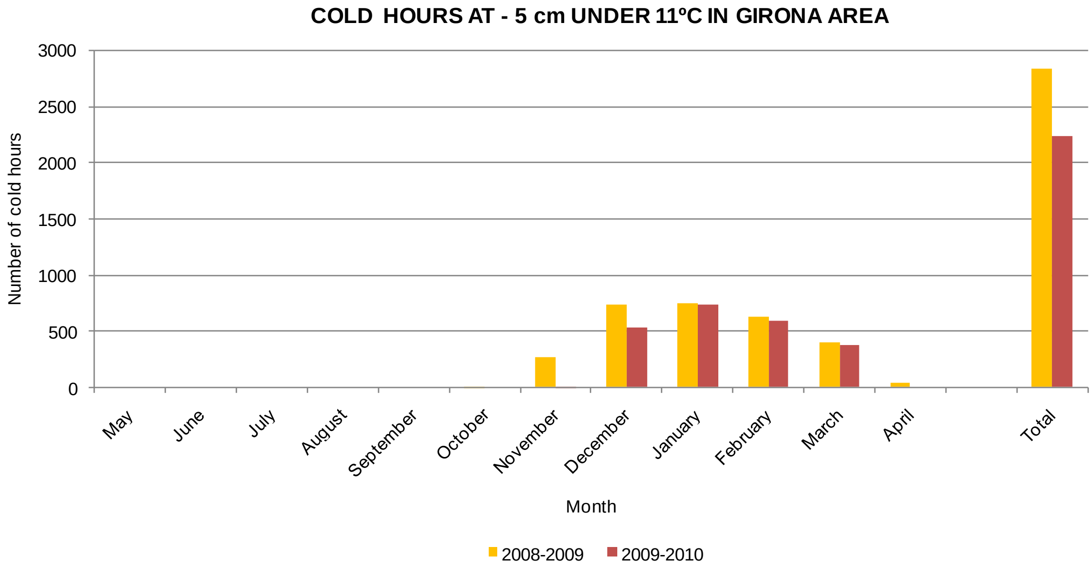


Figure 14. Number of hours registered under the development threshold of pupa, 11ºC at 5 cm below surface in the two years studied.

3.3 ADULTS TRIAL

The results of this trial suggested that *C. capitata* is unable to survive throughout the winter season in the adult stage in the Girona fruit growing area. Adults subjected to external conditions remained immobile inside the evolutionary cages, resting on the mesh walls or in the iron-clad corners, but adults inside the chamber were more active.

Under natural winter conditions in Girona, adult flies survived an average of 8.43 to 9.88 days in the first study period (starting on mid December) and 28.45 to 30.24 days in the second (starting on mid November), but in chamber conditions, they survived an average of 15.5 days and 12.87 days, respectively. The maximum survival period in natural conditions was 11 days in the first winter and 35 days in the second and in controlled conditions, the figures were 29 and 38 days, respectively (Table 2).

Table 2. Number of individuals used in each cage, the survival average with its standard error, the maximum number of days survived and the maximum age achieved.

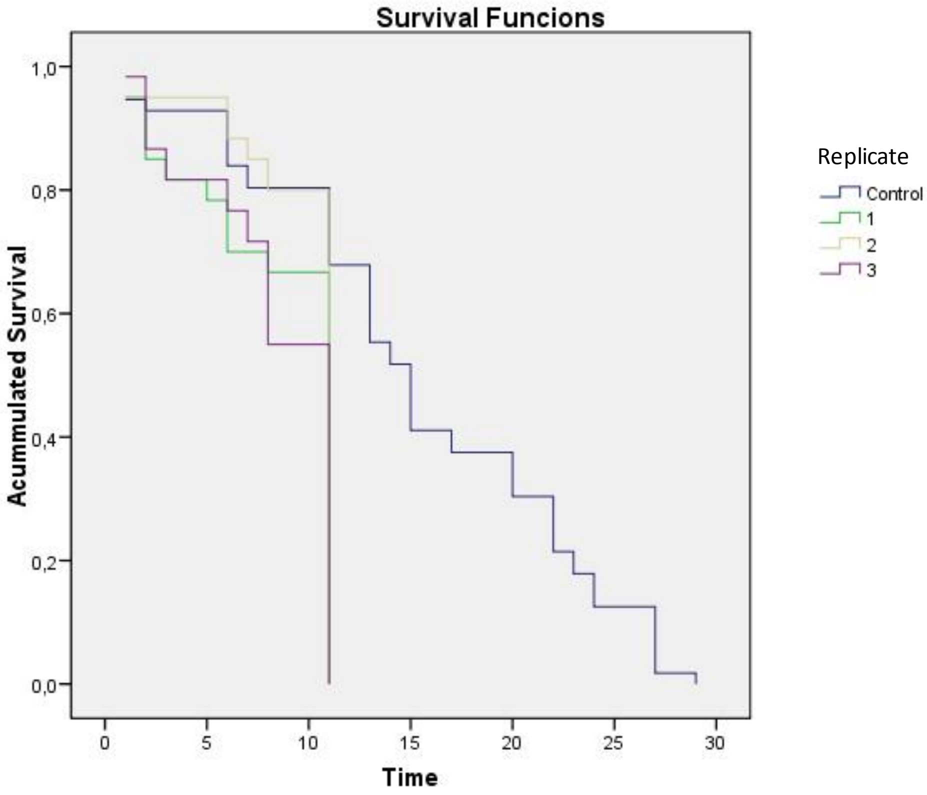
YEAR OF STUDY	REPLICATE	Nº OF ADULTS	SURVIVAL AVERAGE ± S.E. (DAYS)	MAXIMUM DAYS OF SURVIVAL	MAXIMUM AGE ACHIEVED
2008	1	60	8.62 ± 0.47	11	18
	2	60	9.88 ± 0.33	11	16
	3	60	8.43 ± 0.44	11	18
	Control	56	15.5 ± 1.02	29	34
2009	1	54	30.24 ± 0.85	35	41
	2	45	29.04 ± 0.78	32	38
	3	55	28.45 ± 1.11	35	41
	Control	58	12.87 ± 1.30	38	43

Graphs of the accumulated survival rates over the study period were drawn, in order to ascertain the likelihood of survival. Individuals subjected to control treatments lived longer than the specimens outdoors in both cases (Figure 15). The Log-rank test indicated with 95% of confidence, that there were significant differences between the survival of individuals from the control and the other three cages in both years. Over the entire study between 2008 and 2009, there were significant differences between the group of individuals from replicates 2 and 3. In the 2009 study, there were also differences between replicates 1 and 2 and between replicates 2 and 3 (Table 3).

Table 3. Comparison of the survival average in pairs, chi squared statistic and its significance (Log Rank test, $P < 0.05$).

YEAR OF STUDY	REPLICATE	REP. 1		REP. 2		REP. 3		CONTROL	
		χ^2	SIG.	χ^2	SIG.	χ^2	SIG.	χ^2	SIG.
2008	**REP. 1**	-	-	3.19	0.074	0.92	0.338	40.23	0
	REP. 2	3.19	0.074	-	-	8.02	0.005	34.41	0
	REP. 3	0.92	0.338	8.02	0.005	-	-	42.26	0
	CONTROL	40.23	0	34.41	0	42.26	0	-	-
2009	**REP. 1**	-	-	13.27	0	0.6	0.439	46.92	0
	REP. 2	13.27	0	-	-	5.57	0.018	35.86	0
	REP. 3	0.60	0.439	5.57	0.018	-	-	36.60	0
	CONTROL	46.92	0	35.86	0	36.60	0	-	-

TRIAL WINTER 2008 - 2009



TRIAL WINTER 2009

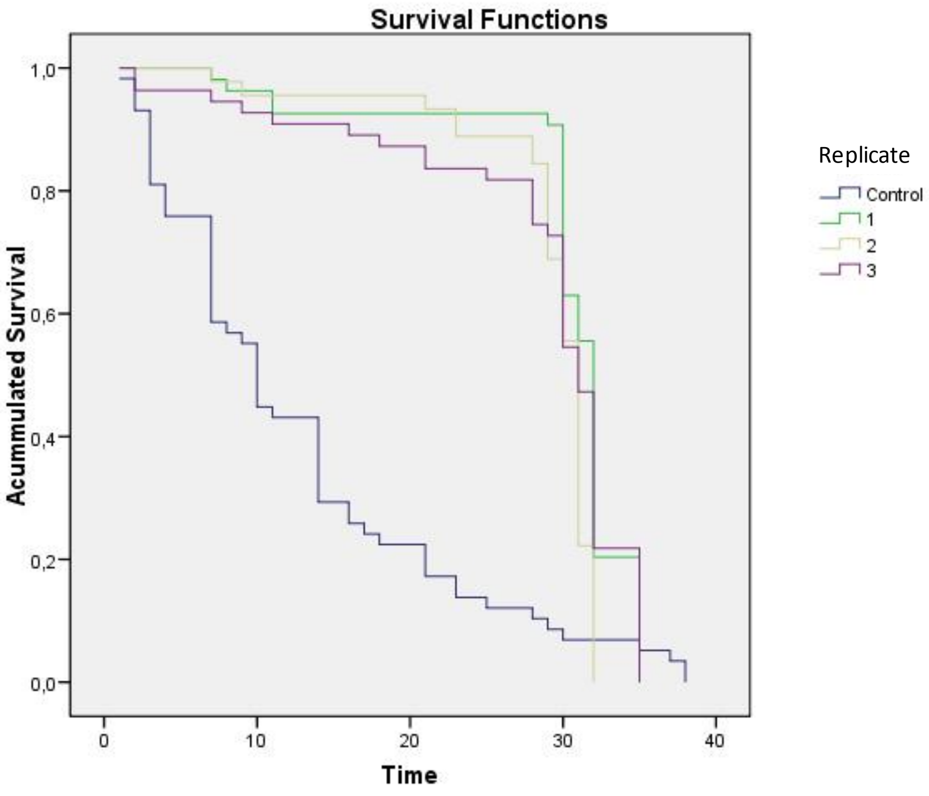


Figure 15. Survival function of all three replicates installed under natural conditions and the control installed in the chamber for each studied period.

The temperature and relative humidity registered in the evolutionary cages using data-loggers were very similar to those recorded at the nearby meteorological station, with maximum differences of ±1ºC.

Line graphs showed survival percentages in each outdoor cage and the maximum and minimum temperatures recorded by data logger inside the plot. The absolute maximum temperature recorded was 18.8ºC in the first year (Figure 16) and 22.3ºC in the second (Figure 17). The absolute minimum temperature recorded was - 2ºC in the first winter season (Figure 16) and - 8.1ºC in the second (Figure 17). The average temperature recorded inside the plot was 6.89ºC in the first period studied (Figure 16) and 8.38ºC in the second (Figure 17).

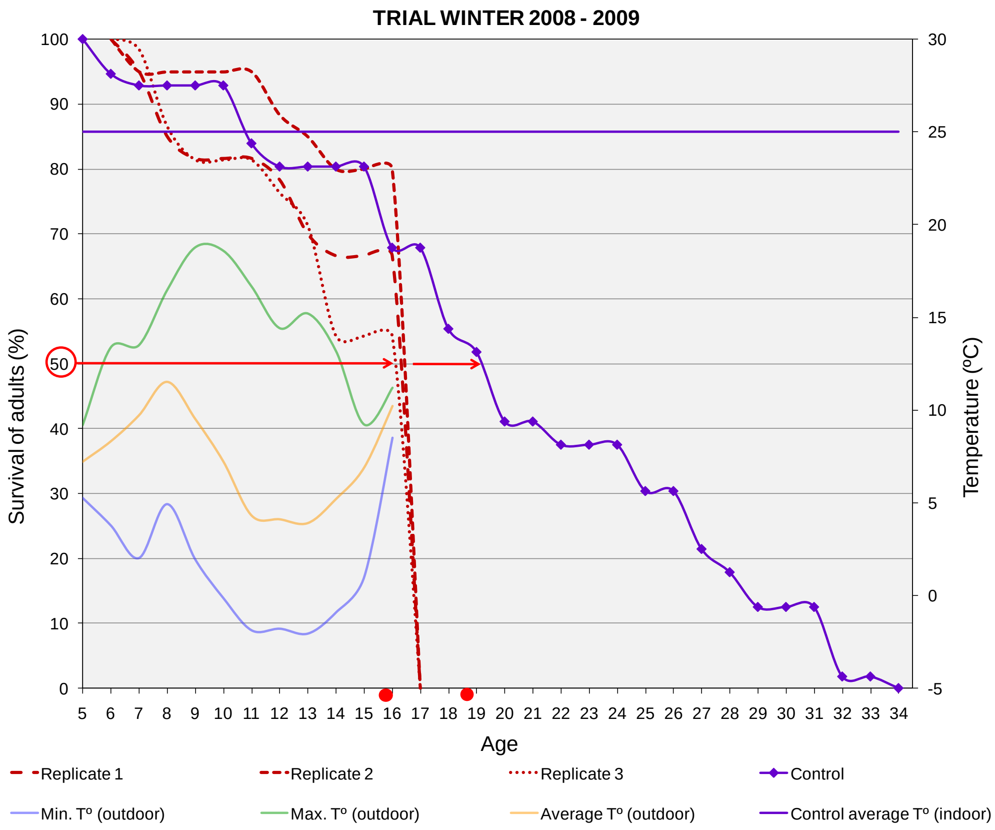
Relative humidity registered over the study periods in the field cage was 47.54 to 100% in 2008 and 40.38 to 62.49% in 2009.

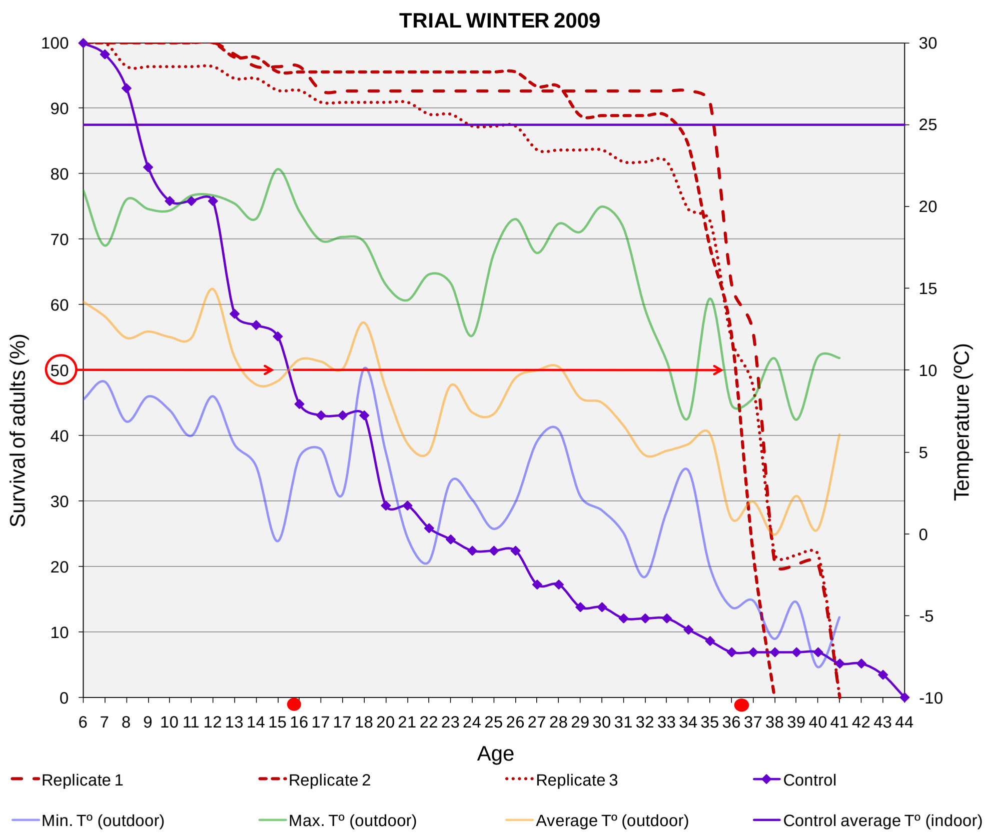
Accumulated rainfall over the first study period was 67.2 mm, with a maximum daily rainfall of 54.6 mm recorded on 26th December, 2008. In the second study period, these figures were 7.6 mm and 2.6 mm, respectively.

The most common wind direction registered over the two periods studied was Northerly. It was recorded on 42% of the days in the first trial period and on 39% of the days in the second. However, hourly records showed that between 13h on 26th December and 8h on 27th December in the first study year, an Easterly wind blew with a maximum speed of between 48.6 km/h and 60.5 km/h. Rainfall recorded over these two days was 62.4 mm. This climatic phenomenon of strong winds from East accompanied by high rainfall coincided with the death of all remaining live individuals in the three cages, which were 66.67%, 80 and 54.24%, respectively.

In the second year studied, on the day after the absolute minimum temperature was recorded (- 8.1ºC), 20.4 and 21.8 % of the remaining live individuals from replicates 1 and 3 perished.

The age of flies at the point at which 50% mortality was achieved (equivalent to 50% survivorship) was calculated. From these figures it was possible to compare the age of the flies at the point of 50% of mortality in the cages installed outdoors and indoors for each trial. In the first year, 50% mortality of individuals was achieved when flies subject to external conditions were between 16 and 17 days old, while in chamber conditions they were 19 days old (Figure 16). In the second trial, a greater difference was observed between the age of flies at 50% mortality in natural conditions and those in controlled conditions. The age of those outdoors was 36 to 37 days and the ones indoors 15 days (Figure 17).





Figures 16 and 17. Adult survival depending on their age over the 1st and 2nd trial and Tº registered within the plot and under chamber. It is indicated the 50% of mortality for the individuals and their age.

In the first year, between 16th November and the death of the last individual, the maximum number of cold hours below 9ºC tolerated by adults was 173 hours, while in the second winter season, from 16th October to the end of the trial this value was 464 hours.

The value of MSA should be higher than 0.5 to be able to do a factor analysis. The values of MSA were 0.6 in the first year studied and 0.56 in the second one. Therefore, the PCA on correlations was carried out.

In the factor analysis of the first year the first three eigenvalues explicated the 96.69% of variability of the data, while in the second year they explicated the 95.26% (Table 4).

Table 4. Eigenvalues, percentage of variability and cumulative percentage of variability in the two studied years.

YEAR OF STUDY	NUMBER	EIGENVALUE	PERCENT OF VARIABILITY	CUMULATIVE PERCENT OF VARIABILITY
2008 - 2009	1	3.718	61.96	61.96
	2	1.335	22.25	84.21
	3	0.749	12.48	96.69
	4	0.143	2.38	99.07
	5	0.052	0.86	99.93
	6	0.004	0.07	100
2009	1	3.067	51.12	51.12
	2	1.724	28.74	79.86
	3	0.924	15.40	95.26
	4	0.163	2.72	97.97
	5	0.117	1.94	99.92
	6	0.005	0.08	100

In the winter of 2008 - 2009 the first eigenvector gave higher weight to the variables of minimum and average temperature and in a lower extent to the maximum temperature. In the second eigenvector the weight of survival and rainfall were higher. In the third eigenvector the rainfall showed again the higher weight (Table 5).

Similar results were obtained in the second year of study, regarding the first and third eigenvectors. Nevertheless, the second eigenvector gave higher weight to the age of flies and the survival.

Table 5. Eigenvector for each factor in the two studied years.

YEAR OF STUDY	FACTOR	EIGENVECTOR		
		1	2	3
2008 - 2009	Age	0.416	-0.444	-0.217
	Survival	-0.323	0.582	0.401
	Rainfall	-0.159	-0.582	0.776
	Minimum Tº	0.488	0.139	0.289
	Maximum Tº	0.471	0.282	0.115
	Average Tº	0.487	0.164	0.304
2009	Age	-0.100	-0.711	0.175
	Survival	-0.269	0.625	-0.193
	Rainfall	-0.108	0.258	0.959
	Minimum Tº	0.557	0.008	0.094
	Maximum Tº	0.524	0.189	-0.030
	Average Tº	0.566	0.039	0.057

4 DISCUSSION

4.1 OVERWINTERING OF LARVAE AND PUPAE IN NATURALLY INFESTED APPLES

The pre-adult development of medfly depends on temperature but also on the host fruit (Rigamonti, 2004a) and the cultivar host. For instance, the development period on peaches is 3 to 6 weeks on average, while on apples it is 5 to 9 weeks (Rigamonti, 2004a). All fruits involved in the present study were collected in the same plot and the total number of larvae found in ‘Golden Delicious’ apples was much higher than those found in ‘Granny Smith’, thus the first variety was more susceptible than the second one. This difference in the rate of infestation of both cultivars has been described in other studies (Romani, 1997) (Papadopoulos et al., 2001a).

The duration of larval development in winter may be critical because the longer an individual remains inside a host fruit as a larva, the less the chance it has of being exposed to lethal low temperatures as a pupa (Papadopoulos et al., 1996). The period between the beginning of this trial and the emergence of mature larvae depended on the age of the individuals in the moment of picking the fruits, which was unknown. Nevertheless, the average length of this period was similar for both ‘Golden Delicious’ and ‘Granny Smith’ apples when they were kept under controlled conditions. It has been found that low temperature increases the development time of immature stages (Segura et al., 2004), as it was observed in the present study, judging from the date of the emergence from fruits under natural winter conditions and from fruits maintained at 25ºC. Outdoors, larval development from fruit sampling until their exit from the fruit took a maximum of two and half months and in a winter trial on Crete Island (Southern Greece) a shorter period was observed from fruit sampling to larval exit (up to 2 months) (Mavrikakis et al., 2000).

The fact that the last mature larva jumped on December 20th in 'Golden Delicious' and on November 22nd, in 'Granny Smith' shows the importance of collecting and destroying the non commercial fruits of all cultivars in a large area during winter, thus avoiding the development of larvae over the last part of the year. Removal of infested fruits in this way has been shown to result in very low population levels the following spring (Papadopoulos et al., 2001a). Similar findings were described in a study performed in North Italy, where not a single larva was able to survive inside apples after the end of January. All died in the first period of intense cold (under 0ºC for more than a month) (Rigamonti, 2004b).

The average length of the mature larva stage from the emergence of fruit until pupal instar was similar in both cultivars studied, although it was slightly lower under natural conditions for both varieties.

Larvae mortality in both conditions could be due to several factors, being one of them the stress originating from up to 24 hours spent in water, from when they jumped until they were collected. Larvae mortality of *C. capitata* associated with their immersion in water for over 24 hours has been recorded to be as high as 21% (Eskafi and Fernández, 1990). The low levels of mortality found in the present study both indoors and out indicated that larvae did not spend too long immersed. High pre-pupal mortality in outdoor conditions has been reported by other researchers working on 'Golden Delicious' and 'Granny Smith' cultivars (Papadopoulos et al., 1996).

The differences between the average number of larvae per kg of fruit registered in the chamber and outdoors for both varieties could be due to climatic factors, including temperature and relative humidity, which would have a negative impact on the survival of larvae. Different results were obtained using wild apples, where from one hundred newly-emerged first instars disposed on one gram of the host, not a single larvae survived (Krainacker et al., 1987). Despite these results, apples are considered to be an important host for medfly, as was shown in a study where the percentage of samples containing infested fruits was higher in apples (with maximum of 22 and 19 larvae per fruit for 'Golden Delicious' and 'Granny Smith' varieties), followed by ornamental persimmons, figs, pears and peaches (Papadopoulos et al., 2001a).

The duration of the pupa stage observed at 25ºC with controlled R.H. was comparable in 'Golden Delicious' and 'Granny Smith' cultivars (11.63 and 12.08 days) and coincided with that described in a study under similar conditions (11.5 days) (Shoukry and Hafez, 1979). The pupal stage can be prolonged several months in low temperatures and with minimum humidity (Aluja, 1993) (Mavrikakis et al., 2000). The average duration of the pupa stage in 'Golden Delicious' fruits was 2.5 times greater in natural conditions than indoors, which suggests that climatic factors such as temperature or R.H. are parameters that induce a slowing of development at the pupa stage. In a study performed in Israel, it was also argued that medfly overwintering in late apple varieties slowed down their development rate during the winter months (Israely et al., 1997). In another study the mean developmental period of the different stages of medfly was longer at the minimum temperature observed (14ºC) than at higher ones (18ºC, 22ºC and 26ºC), which confirms the deceleration of growth at lower temperatures (Grout and Stoltz, 2007). As demonstrated by a classic study (Davidson, 1944), the duration of the pupa development period in insects can vary at constant temperatures, i.e. in *Drosophila melanogaster* Meigen the lower the temperature, the longer the duration of the pupal stage. In some insect species the number of instars can be also increased by low temperature (Denlinger and Lee, 1998), thus prolonging the development period.

The later larvae leave the fruits to pupate, the higher their survival rate (Papadopoulos et al., 1996). This fact was confirmed in the present study: as winter advanced, the development time of the pupa stage became extended, thus enhancing the likelihood of survival. These results conform to the observations found in Crete, where the duration of the pupal stage at field temperatures was close to two months for those pupae formed between December and February and reduced progressively to 10 - 20 days for those pupae formed in May (Mavrikakis et al., 2000).

Pupae mortality in the present study was 38.71% under controlled conditions and in a study on the biology of medfly conducted in laboratory using also 25ºC and 30% R.H., 60 % R.H. or 90 % R.H., pupae mortality was 42, 20 and 25%, respectively (Shoukry and Hafez, 1979). Therefore, pupae mortality could have been influenced by relative humidity and by the fact that in the present study, individuals suffered stress owing to the low temperatures which occurred throughout the egg stage and the first larvae instars, until fruits were brought to the chamber.

The difference found between mortality registered at the pupa stage under natural conditions and in controlled ones could be due to the effect of the very low temperatures registered in the area. The results of the present study back up the findings of other authors, who reported that prevailing low winter temperatures resulted in a very high mortality in the immature stages (Papadopoulos et al., 1996) (Segura et al., 2004). Nevertheless, the slow development rate of medfly larvae inside apples and the good condition of the fallen fruits over the winter period allowed larvae which were oviposited in autumn to survive the winter months (Papadopoulos et al., 1996). The extended stay inside the fruit guarantees the larvae better conditions than the soil does for the pupae (Papadopoulos et al., 1996). This corroborates the present findings: outdoors the percentage of larval mortality of individuals emerging from both varieties was much lower than the percentage of pupa mortality.

The ectoparasitoid *M. acasta* detected in the study is the only species of this genus native to Europe and has been reported on 28 groups of species as hosts, including some Diptera, although none of them is a fruit fly (Gonzalez et al., 2004a) (Gonzalez et al., 2004b). This is a regular parasitoid of bees and in the Girona region at blossom time, it is usual to install bee panels or hives which help in the pollinating process. Therefore, the origin of this parasitoid could be one of these colonies, which had eventually become a parasite of the medfly, providing a possible cause of mortality of individuals at the pre-adult stages.

The percentage of adult emergence was much higher in the chamber than under natural conditions. The low levels of adult survival in both conditions, a maximum of 15.06% in exterior ones and of 57.86% in controlled ones could be due to the stress suffered in the larval stage due to the time spent under water. If further studies were to be performed, it would be preferable to pick the larvae as they emerge from the fruit using solid surfaces such as cellulose absorbent paper as at the end of the present trial, or sterilised sand, although ripened fruits produce leaching which makes the collection process difficult.

Under natural conditions the emergence of adults from 'Golden Delicious' fruits lasted 67 days longer than in controlled ones. This would be due to the accumulation of longer development time of larvae and pupal stages. The last emergence of adults reared in natural conditions inside 'Golden Delicious' apples, took place in mid January and the ones reared inside 'Granny Smith' occurred at the end of December, but to understand the activity of adults in winter, further studies are required using methods developed in the third trial of the present study.

Of the larvae found in this study, no adult emerged after 21st March, the first day of spring; so it was not possible to prove that adults found in spring or summer were coming from fruits infested in the winter season. Similar results were found in an overwintering study conducted in 1995, where all adults were collected from pupae formed in spring (Papadopoulos et al., 1996). However, very different results were found in other trials carried out in same area over the previous year. That study reported that 1.9% of 1,528 larvae obtained in winter 1994 developed into adults the following spring, and of 211 larvae obtained in spring (after spending the winter inside fruits) 14.2% reached adulthood (Papadopoulos et al., 1996). In the Northern Mediterranean coasts, therefore, medfly overwinter inside apple fruits as first and second larval instars, which makes it highly likely that their development is slowed, perhaps even for several months, without lethal consequences, whereas older instars have a tendency to abandon the fruits to pupate. All other stages (grown larvae, pupae, adults and eggs) perish during winter (Papadopoulos et al., 1996) (Papadopoulos et al., 1998). In another study, a small overwintering larval population was present within citrus fruits, but because there was a high abundance of these fruits over winter, this contributed to a rise in the medfly population the following spring (Katsoyannos et al., 1998). The existence of a narrow period in which oviposition must occur to maximize the possibilities of reaching adulthood in apple hosts in spring, is from late autumn to early winter in conditions such as those in Northern Greece (Papadopoulos et al., 1996) and at the beginning of winter in a climate such as that in Valencia (Del Pino, 2000). Further research would be necessary to verify if there is also an optimal period for oviposition which enhances the survival of larvae until spring-summer in the Girona area.

The number of medfly adults found per kg of ‘Golden Delicious’ fruits was six times higher in winter conditions than for those indoors. The apple variety plays an important role in the number of medfly adults per kg of fruit, as has been observed under controlled conditions using ‘Golden Delicious’ fruits (34.85 adults/kg) and ‘Granny Smith’ fruits (1.59 adults/kg). Having used the same controlled environment for both cultivars, this strengthened corroboration of the susceptibility of the different cultivars. In another study performed two decades ago, wild apple fruits were tested under controlled conditions (19ºC to 24ºC), registering high loads of individuals (79.02 adults/kg) (Liquido et al., 1990).

In spite of the similar percentage of males and females emerging from ‘Golden Delicious’ fruits in both kinds of conditions, contradictory results were found in a study performed in Australia, where a higher proportion of females was detected after the overwintering period. They suggested that females may move out of overwintering sites earlier than males or that females are more resilient to winter conditions (Broughton and De Lima, 2002).

Temperature drops over winter have been shown to cause a dramatic decrease in the overwintering population in Mediterranean areas (Papadopoulos et al., 1996). Low temperature could be also associated with the increase in the development time for immature stages and this in turn could affect adult population levels (Katsoyannos et al., 1998) (Papadopoulos et al., 2001a) (Segura et al., 2004). Monthly minimum absolute temperatures registered in the experimentation area remained below zero degrees from November 2007 to January 2008. As larval development stops at 5ºC (Shoukry and Hafez, 1979) (Vargas et al., 1996) and the threshold for pupae development is 11.2ºC (Duyck and Quilici, 2002), low temperatures could be a determinant factor in the survival of the different stages of medfly in Girona.

4.2 PUPAE TRIAL

In the overwintering of pupae trial in Girona area, no adult emerged from the pupae in winter conditions in either of the two years in question. These results support previous studies carried out in Northern Italy: in 1995 and 1996 no pupae emerged after being installed 5 - 15 cm below the soil surface (Romani,

1997) and in 2000 and 2002 pupa were unable to survive in natural winter conditions (Rigamonti, 2004b). High pupal mortality has been also observed in other fruit fly species, including *Dacus tryoni* (Froggatt), which, towards the end of the season, showed a high pupal mortality and a cessation of pupal production due to diminution and eventual disappearance of larval food (Bateman and Sonleitner, 1967).

The average length of the pupa stage observed at 25ºC with controlled R.H. was 11 days, which coincides with the findings of the trial performed the previous year in the Girona area (section from above) and with another study carried out under similar conditions more than thirty years ago (Shoukry and Hafez, 1979).

Despite the non-survival of pupae under natural conditions, the percentage of emergence inside the chamber corroborated the viability of the population used in both trials. In another study, the average survival rate for medfly maintained at 25ºC with 80 ± 10% R.H. was 79% (Duyck and Quilici, 2002). These results are of the same order as the maximum survival rate observed in the trial conducted in 2008 - 2009, though the maximum survival rate recorded in 2009 - 2010 was slightly lower, being 76.67%. In another overwintering trial, a higher percentage of emergence was found among pupae maintained under laboratory control at 25ºC (87.3%) (Papadopoulos et al., 1996).

At the end of the trial, 60% of the pupae were missing from the glasses maintained under natural conditions. The potential predators found were individuals from the species *F. cunicularia*, which are mainly predators (Collingwood, 1979) (Sanders and Platner, 2006). Although these ants have been found in citrus orchards from Tarragona (Palacios et al., 1999) and Portugal (Vera and Leitão, 2008), they have not been recorded as predators of any fruit fly. Nevertheless, a species from the same genus, *Formica rufibarbis* Fabricius was found to be a predator of medfly, being the third most common species of ants collected in citrus groves in Valencia (Urbaneja et al., 2006). The existence of some pupae with holes and the presence of these potential predators inside several glasses in both years indicated that predation should be taken into account in the evaluation of pupae survival in Girona area. Nevertheless, further research is required in regard with this subject.

Because mature larvae abandon the fruit and drop to the ground to pupate, soil humidity has a strong and direct effect on the pupal development of medfly (Eskafi and Fernández, 1990). Although it has been shown that this species is relatively tolerant to desiccation (Shoukry and Hafez, 1979) (Duyck et al., 2006), checks made on pupae recovered after the trial, showed that a low percentage had dried up in both, natural conditions and in the chamber.

Water loss during a lengthy pupal period at low temperature in soils with low water retaining capacity during the dry season cause pupal mortality (Eskafi and Fernández, 1990). Soil structure is also a reason why pupae fail to emerge after winter (Romani, 1997). The present study was carried out in the top five centimeters of the soil surface, taking into account the results obtained in pupal trials in which 90 - 95% of the pupae were concentrated in the top five centimetres, 70% of them being in the top two centimetres (Rigamonti, 2004b). The soil in the studied plot had a sandy-loam texture with a high sand content, which has very low water holding capacity. Periods of low temperature followed by several days without rainfall could have increased the mortality of some pupae.

The soil's relative water content (SRWC) has an effect on pupal development and the survival of other fruit flies, including *Bactrocera tau* (Walker): a high SRWC results in pupae mortality (Li et al., 2009). Anoxia can be a cause of pupae death during the rainy season in soils with high bulk densities (Eskafi and Fernández, 1990). The mean survival of pupae is deemed to be 25% after one day in unaerated water and 3.75% after two days (Eskafi and Fernández, 1990) but in other study it has been demonstrated that no pupae of medfly survived more than six hours of immersion in water (Duyck et al., 2006). Although the soil in the tested area was sandy-loam, the high level of rainfall registered in a few days over the study period of both years could have produced the anoxia of some of the pupae.

Atmospheric humidity strongly influences the survival of pupae of medfly (Romani, 1997) (Duyck et al., 2006). Average of relative humidity registered in the study plot was over 75% in both crop seasons and evidence from other studies would relate it with a survivorship of 80% of pupae (Duyck et al., 2006). Less than 40% of medfly pupae can survive at 30% R.H. (Duyck et al., 2006) but throughout the present study, only one period of nine hours and another of three were recorded each year with R.H. below 30%, with minima as low as 25%.

In both winters the cold hours below the threshold for pupae in outdoor conditions was higher than in the top part of the soil, due to the buffer effect of the ground. The number of cold hours registered in the area (more than 2,200), was long enough to prevent the development of pupae at soil level. Ancient studies showed that medfly pupae cannot exist more than eight days at a soil temperature of 2ºC (Feron and Guennelon, 1958). Climatic data from the present work showed that in 2008 - 2009, minimum temperature registered at subsoil level did not reach 2ºC on any occasion, the minimum found being 3.7ºC. In the second winter season studied, at 5 cm below the surface, there were two periods with minimum temperatures in a range close to 2ºC, being none of them more than eight days: one period of two days (1.8ºC and 2.2ºC) and another period of three days (2.2ºC, 2.1ºC and 1.5ºC). Nevertheless, no pupae survived the winter, indicating that factors other than soil temperature were also involved in the mortality.

4.3 ADULTS TRIAL

Adults of medfly are unable to survive low winter temperatures in some Mediterranean areas including Greece (Papadopoulos et al., 1996), as was shown in an early study in which was confirmed that they survived less than 48 hours at 0ºC (Cheikh, 1967). This was corroborated in the present overwintering study with adults from a population native to Girona. Adults survived winter conditions in this area from mid to late December and from mid November to the third week of December. This intervals were included in the period of survival of adults from an overwintering trial carried out in Northern Italy, where minimum temperatures between 5ºC and - 2ºC were recorded (Romani, 1997).

Some factors are related to the insect’s resistance to cold, which is affected by its microhabitat, that determines the availability of moisture, the developmental temperature and matters such as humidity and desiccation tolerance (Danks, 2006).

During cold and temperate winters, most species are inactive, leading to a seasonal state of quiescence, dormancy or even diapauses that vary with species and circumstances (Meats, 1989) (Danks, 2006). It has been shown that, at lower latitudes in temperate regions, populations of certain Tephritid species (e.g. *Eurosta solidaginis* (Fitch)) are less cold tolerant than those from higher latitudes (Denlinger and Lee, 1998). Nevertheless, in some of the Southern Mediterranean areas, a small number of medfly adults might be active during winter (Papadopoulos et al., 2001a) (Martínez-Ferrer et al., 2007). In other regions, including Valencia, adults are able to survive winter, at temperatures lower than 0ºC (Del Pino, 2000). In Crete (Southern Greece) adults survived all winter with minimum temperatures between 1ºC and 4.5ºC (Mavrikakis et al., 2000) and in Italy some medfly females survived throughout winter to lay eggs in spring (190 - 250 days after emergence), thus assuring new populations for the next season (Rigamonti, 2004b). In the Girona study of two years all individuals used in the trials died during winter.

The significant differences found in both years between the survival of individuals from the control and the other three replicates demonstrated that winter conditions in the Girona area affected the mortality of the adult medfly population. In the first year, the average survival rate of control individuals was significantly higher than for the three replicates. On the other hand, in the second year the average survival rate of control individuals was significantly lower. The differences between these results were probably due to the time at which each study was carried out. The first study began in mid November, 2008 and the second in mid October, 2009 but the difference could also be explained by the heavy storm that occurred on the day prior to the end of the first trial.

Significant differences were also found between survival from replicates 2 and 3 of the first year and between replicates 1 and 2 and replicates 2 and 3 of the second year. These variations could be related to physical differences between the second compartment and the rest of the plot. Temperature and relative humidity were recorded in each compartment and minimum variations were found, so it could be argued that the wind regimen could also be one of the limiting factors, in spite of the protection mesh structure present in the Northern part of the plot. Over the two periods of the study, a North wind was recorded for an average of 40% of the days and the orientation of the plot was also to the North. Even though a thicker mesh was used as reinforcement in the North face of the plot, it is possible that the intensity of the wind inside each compartment differed enough to affect the survival of the adults inside.

The temperature threshold for population growth is 12ºC to 35ºC (Vera et al., 2002) and maximum temperatures in both years were always below the upper limit. However, minimum temperatures in the studied periods sometimes fell below the lower threshold. Minimum temperatures from - 2ºC to 8.5ºC were recorded in the first period studied and from - 8.2ºC to 10.1ºC in the second, which almost certainly affected the optimum development of the population growth.

There is a high variability in the severity of the minimum temperature and the duration of exposure to low temperatures in the temperate zone (Turnock and Fields, 2005). It has been shown that, low minimum temperatures slow down adult activity (Romani, 1997), but active behaviour would probably expose vulnerable individuals to dangerous conditions (Danks, 2006). A rise in temperature could activate their development and thus cause their death. This hypothesis was checked in the second year, when the minimum temperature on 14th December, 2009 registered 3.9ºC. On this date the survivorship rate for adults was 92.6, 84.4 and 74.5% in each replicate. Four days later the absolute minimum temperature dropped to - 6.4ºC, which coincided with a marked drop in the survival rates to 20.4, 0 and 21.8%. These results supported the earlier proposition, but further studies must be performed in order to calculate more precisely the relationship between minimum temperatures, sudden rises and the mortality of adults at field level. Similar results were found in the weekly survival rates of adults of the fruit fly *D. tryoni*, in an overwintering site. Mortality was related to the minimum temperatures and when sub-zero temperatures occurred, mortality rate increased (Fletcher, 1979).

A recent study has focused on the minimum critical thermal limit (Ct_{min}) for medfly, the temperature at which each individual insect loses co-ordinated muscle function, and consequently the ability to respond to mild stimuli

(Nyamukondiwa and Terblanche, 2009). Adults exposed to this threshold recovered, so it was not immediately lethal. Depending on age of the flies Ct_{min} was 5.4ºC to 6.6ºC (Nyamukondiwa and Terblanche, 2009). Taking into account these Ct_{min} and the minimum temperatures registered in Girona over both studied periods (- 2ºC and - 8.2ºC), it is possible that the population from the study would have lost co-ordinated muscle function, in which case they would have been susceptible to a rapid demise.

In regard to the other climatic factors, the effect of rainfall on the population of medfly, has been related to a decrease in adult captures on rainy days and an increase a few days later, because flies are generally inactive during periods of moderate to heavy rainfall (Christenson and Foote, 1960) (Cayol and Causse, 1993) (Appiah et al., 2009). In the current trial the mortality of adults was observed to be affected by rainfall. In the first year, rain fell for only a few hours but this had a negative effect on adult survival. The highest daily rainfall registered in the first studied period took place on the day before the end of the trial regarding outdoors, as part of the storm on 26th December, 2008. Therefore, in spite of the mesh that covered the plot and the slight inclination of the evolutionary cages, the high level of rainfall drowned all the remaining adults. In the second study season the mesh placed in the bottom of the evolutionary cages prevented the individuals from death by drowning.

The mean age achieved by the flies under controlled conditions was 21.5 days in the first year and 18.9 days in the second. These were lower values than those recorded in a survival study of medfly at 25ºC, in which males survived a mean of 36 days and females 31 days (Shoukry and Hafez, 1979). They were also lower than those in other biology study where the average female longevity at 25ºC was 35 days (Boller, 1985). These differences in the average longevity under controlled conditions could be due to other factors present in the chamber, such as the relative humidity and the photoperiod, or to the cohort of the flies used (being F2 and wild ones in the Girona trials).

Estimates of the time taken to reach 50% mortality have been used to describe the level of the insects' cold hardiness and non-freezing cold injury (Turnock and Fields, 2005). The age at which half of the population used in the trials had died was very similar in the three replicates of each year studied, but there were differences between the two years, the first being more than twice that of the second year. The results in the first year were related to the high rainfall and, maybe, strong winds of the storm which occurred the day before the point at which half of the population had died. Values corresponding to control flies from both years differed by only 4 days, showing homogeneity in the population used in the study.

The relation between survival of adults and the number of accumulated cold hours below 9ºC in the first period studied was also influenced by the high rainfall and strong winds of 26th December, 2008. Therefore, flies from the second year supported more than twice cold hours than in the first year.

The statistical analysis of PCA showed that in the first winter the temperature and rainfall were the main factors affecting the survival, while in the second winter the temperature and the age were the most important. Therefore, it was demonstrated that the strong rainfall of the first year affected the survival of flies, while in the second year this factor did not appeared to be of the same importance.

5 CONCLUSIONS

Stages of larvae and pupae of medfly collected from infested apples survived the natural conditions of late autumn and early winter in the Girona fruit growing area but not through the entire winter period. Larval and pupa stages maintained in winter conditions developed more slowly when compared with individuals reared in controlled ones. Adults continued to emerge until mid January, so it was not possible to prove that adults found in the following spring-summer came from fruits infested in winter.

No adult medfly emerged from pupae which spent all winter under natural conditions in either of the two years analyzed, due to several factors at subsoil level, i.e. the high number of cold hours below the threshold for pupae development, desiccation of pupae caused by some periods of low temperature followed by several days without rainfall or anoxia caused by high levels of rainfall.

Medfly adults were unable to survive the entire winter season in the Girona fruit growing area in either of the two years studied. Climatic conditions including daily temperature and high level of rainfall were involved in the mortality of adults during winter.

6 REFERENCES

Aluja, M., 1993. The study of movement in Tephritid Flies: Review of concepts and recent advances. Fruit Flies: Biology and Management, pp. 105-113.

Appiah, E.F., Afreh-Nuamah, K., Obeng-Ofori, D., 2009. Abundance and distribution of the Mediterranean fruit fly Ceratitis capitata (Diptera: Tephritidae), in Late Valencia citrus orchards in Ghana. International Journal of Tropical Insect Science 29**,** 11-16.

Bateman, M.A., Sonleitner, F.J., 1967. The ecology of a natural population of the Queensland Fruit Fly, Dacus tryoni. I. The parameters of the pupal and adult populations during a single season. Australian Journal of Zoology 15**,** 303 - 335.

Boller, E.F., 1985. Rhagoletis cerasi and Ceratitis capitata. Handbook of Insect Rearing 2**,** 135-144.

Broughton, S., De Lima, C.P.F., 2002. Field evaluation of female attractants for monitoring Ceratitis capitata (Diptera : Tephritidae) under a range of climatic conditions and population levels in Western Australia. Journal of Economic Entomology 95**,** 507-512.

Carey, J.R., 1984. Host specific demographic studies of the Mediterranean fruit fly Ceratitis capitata. Ecological Entomology 9**,** 261-270.

Carey, J.R., 1991. Establishment of the Mediterranean Fruit Fly in California. Science 253**,** 1369-1373.

Cayol, J.P., Causse, R., 1993. Mediterranean fruit fly Ceratitis capitata Wiedemann (Dipt., Trypetidae) back in Southern France. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 116.

Cheikh, M., 1967. Etudes des effets de quelques températures basses sur les imagos de la Mouche Méditerranéenne des Fruits Ceratitis capitata Wied. (Diptera-Trypetidae). Faculté des Sciences, Université de Montpellier., pp. 125.

Christenson, L.D., Foote, R.H., 1960. Biology of fruit flies. Annual Review of Entomology 5**,** 171-192.

Collingwood, C.A., 1979. The Formicidae (Hymenoptera) of Fennoscandia and Denmark. Societas Entomologica Scandinava, Fauna Entomologica Scandinava.

Danks, H.V., 2006. Insect adaptations to cold and changing environments. Canadian Entomologist 138**,** 1-23.

DAR, 2008 Agrometeorologia. http://ruralcat.net.

DAR, 2010 Agrometeorologia. http://ruralcat.net.

Davidson, J., 1944. The relationship between temperature and rate of development of insects at constant temperatures. Journal of Animal Ecology 13**,** 26-38.

De Andrés, M., García-Marí, F., 2007. Observaciones sobre la invernación de Ceratitis capitata en la Comunidad Valenciana. Levante Agrícola 385**,** 108-114.

Del Pino, A., 2000. Efecto de factores abióticos y edáficos naturales en el ciclo biológico de Ceratitis capitata (Wiedemann) (Diptera: Tephritidae). Universidad Politécnica de Valencia.

Denlinger, D.L., Lee, R.E., 1998. Physiology of cold sensitivity. Temperature sensitivity in insects and application in Integrated Pest Management., pp. 55-95.

Duyck, P.F., David, P., Quilici, S., 2006. Climatic niche partitioning following successive invasions by fruit flies in La Reunion. Journal of Animal Ecology 75**,** 518-526.

Duyck, P.F., Quilici, S., 2002. Survival and development of different life stages of three Ceratitis spp. (Diptera : Tephritidae) reared at five constant temperatures. Bulletin of Entomological Research 92**,** 461-469.

Escudero-Colomar, L.A., Vilajeliu, M., Batllori, L., 2008. Seasonality in the occurrence of the Mediterranean fruit fly [Ceratitis capitata (Wied.)] in the north-east of Spain. Journal of Applied Entomology 132**,** 714-721.

Eskafi, F.M., Fernández, A., 1990. Larval-pupal mortality of Mediterranean Fruit Fly from interaction of soil, moisture, and temperature. Environmental Entomology 19**,** 1666-1670.

Feron, M., Guennelon, G., 1958. La Mouche des fruits dans la Vallée du Rhône. Jornées fruitières et Maraîchères d'Avignon. Avignon, France 24-25 January 1958. pp. 6.

Fimiani, P., 1989. Mediterranean region. In: Fruit flies, their biology, natural enemies and control. World Crop Pests, pp. 39-50.

Fletcher, B.S., 1979. The overwintering survival of adults of the Queensland Fruit Fly, Dacus Tryoni, under natural conditions. Australian Journal of Zoology 27**,** 403 - 411.

Gonzalez, J.M., Genaro, J.A., Matthews, R.W., 2004a. Species of Melittobia (Hymenoptera: Eulophidae) established in Bahamas, Costa Rica, Cuba, Hispaniola, Puerto Rico, and Trinidad. Florida Entomologist 87**,** 619-620.

Gonzalez, J.M., Teran, J.B., Matthews, R.W., 2004b. Review of the biology of Melittobia acasta (Walker) (Hymenoptera : Eulophidae) and additions on development and sex ratio of the species. Caribbean Journal of Science 40**,** 52-61.

Grout, T., Stoltz, K., 2007. Developmental rates at constant temperatures of three economivally important Ceratitis spp (Diptera: Tephritidae) from southern Africa. Environmental Entomology 36**,** 1310-1317.

Israely, N., Ritte, U., Oman, S.D., 2004. Inability of Ceratitis capitata (Diptera : Tephritidae) to overwinter in the judean hills. Journal of Economic Entomology 97**,** 33-42.

Israely, N., Yuval, B., Kitron, U., Nestel, D., 1997. Population fluctuations of adult Mediterranean fruit flies (Diptera : Tephritidae) in a Mediterranean heterogeneous agricultural region. Environmental Entomology 26**,** 1263-1269.

Israely, N., Ziv, Y., Oman, S.D., 2005. Spatiotemporal distribution patterns of Mediterranean fruit fly (Diptera : Tephritidae) in the central region of Israel. Annals of the Entomological Society of America 98**,** 77-84.

Katsoyannos, B.I., Kouloussis, N.A., Carey, J.R., 1998. Seasonal and annual occurrence of Mediterranean fruit flies (Diptera : Tephritidae) on Chios Island, Greece: Differences between two neighboring citrus orchards. Annals of the Entomological Society of America 91**,** 43-51.

Krainacker, D.A., Carey, J.R., Vargas, R.I., 1987. Effect of larval host on life history traits of the Mediterranean fruit fly, Ceratitis capitata. Oecologia 73**,** 583-590.

Li, X.Z., Liu, Y.H., Wang, J.J., Li, S.B., 2009. Growth, development and water-loss dynamics in Bactrocera tau (Walker) (Diptera: Tephritidae) pupae exposed to determined humidity. The Pan-Pacific Entomologist 85**,** 150-158.

Liquido, N.J., Cunningham, R.T., Nakagawa, S., 1990. Host plants of Mediterranean Fruit Fly (Diptera: Tephritidae) on the Island of Hawaii (1949-1985 Survey). Journal of Economic Entomology 83**,** 1863-1878.

Martínez-Ferrer, M.T., Alonso, A., Campos, J.M., Fibla, J.M., Garcia-Marí, F., 2007. Dinámica poblacional de la mosca de la fruta Ceratitis Capitata en tres zonas citrícolas mediterráneas. Levante Agrícola 385**,** 1-7.

Martínez-Ferrer, M.T., Campos, J.M., Fibla, J.M., 2006. Population dynamics of Ceratitis capitata on citrus in northeast Spain: the influence of adjacent host fruit trees. Bulletin OILB/SROP 29**,** 77-84.

Mavrikakis, P.G., Economopoulos, A.P., Carey, J.R., 2000. Continuous winter reproduction and growth of the Mediterranean fruit fly (Diptera :

Tephritidae) in Heraklion, Crete, southern Greece. Environmental Entomology 29, 1180-1187.
Meats, A., 1989. Acclimation, activity levels and survival. In: Fruit flies, their biology, natural enemies and control. World Crop Pests, pp. 231-239.
Miranda, M.A., Alonso, R., Alemany, A., 2001. Field evaluation of Medfly (Dipt., Tephritidae) female attractants in a Mediterranean agrosystem (Balearic Islands, Spain). Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 125, 333-339.
Nyamukondiwa, C., Terblanche, J.S., 2009. Thermal tolerance in adult Mediterranean and Natal fruit flies (Ceratitis capitata and Ceratitis rosa): Effects of age, gender and feeding status. Journal of Thermal Biology 34, 406-414.
Palacios, R., Martínez-Ferrer, M.T., Cerdá, X., 1999. Composición, abundancia y fenología de las hormigas (Hymenoptera: Formicidae) en campos de cítricos de Tarragona. Boletín de Sanidad Vegetal, Plagas 25, 229-240.
Papadopoulos, N.T., Carey, J.R., Katsoyannos, B.I., Kouloussis, N.A., 1996. Overwintering of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. Annals of the Entomological Society of America 89, 526-534.
Papadopoulos, N.T., Katsoyannos, B.I., Carey, J.R., 1998. Temporal changes in the composition of the overwintering larval population of the Mediterranean fruit fly (Diptera : Tephritidae) in northern Greece. Annals of the Entomological Society of America 91, 430-434.
Papadopoulos, N.T., Katsoyannos, B.I., Carey, J.R., Kouloussis, N.A., 2001a. Seasonal and annual occurrence of the Mediterranean fruit fly (Diptera : Tephritidae) in northern Greece. Annals of the Entomological Society of America 94, 41-50.
Papadopoulos, N.T., Katsoyannos, B.I., Kouloussis, N.A., Hendrichs, J., Carey, J.R., Heath, R.R., 2001b. Early detection and population monitoring of Ceratitis capitata (Diptera : Tephritidae) in a mixed-fruit orchard in northern Greece. Journal of Economic Entomology 94, 971-978.
Rigamonti, I.E., 2004a. Contributions to the knowledge of Ceratitis capitata Wied. (Diptera, Tephritidae) in Northern Italy I. Observations on the biology. Bollettino di Zoologia Agraria e di Bachicoltura 36, 89-100.
Rigamonti, I.E., 2004b. Contributions to the knowledge of Ceratitis capitata Wied. (Diptera, Tephritidae) in Northern Italy II. Overwintering in Lombardy. Bollettino di Zoologia Agraria e di Bachicoltura 36, 101-116.
Rigamonti, I.E., Agosti, M., Malacrida, A.R., 2002. Distribuzione e danni della Mosca Mediterranea della Frutta Ceratitis capitata Wied. (Diptera Tephritidae) in Lombardia (Italia settentrionale). Atti XIX Congresso nazionale italiano di Entomologia. Catania, 10-15 giugno 2002., pp. 579-585.
Romani, M., 1997. Biology, ethology and control of Ceratitis capitata in northern Italy. Informatore Agrario 53, 59-62.
Sanders, D., Platner, C., 2006. Intraguild interactions between spiders and ants and top-down control in a grassland food web. Oecologia 150, 611-624.
Sciarretta, A., Cesare, D., De Salvador, R., Tabilio, M.R., Trematerra, P., 2009. Spatio-temporal distribution of Ceratitis capitata trap catches in an agricultural landscape. Pheromones and other Semiochemicals IOBC/wprs Bulletin 41, 123-129.

Segura, D.F., Vera, M.T., Cladera, J.L., 2004. Fluctuación estacional en la infestación de diversos hospedadores por la mosca del Mediterráneo, Ceratitis capitata (Diptera: Tephritidae), en la provincia de Buenos Aires. Ecologia Austral 14**,** 3-17.

Shoukry, A., Hafez, M., 1979. Studies on the biology of the Mediterranean fruit fly Ceratitis capitata. Entomologia Experimentalis Et Applicata 26**,** 33-39.

Trematerra, P., Salvador, R.d., Cesare, D., Sciarretta, A., 2008. Spatio-temporal distribution of Ceratitis capitata trap catches and integrated pest management in an agricultural landscape of central Italy. Giornate Fitopatologiche 2008, Cervia (RA), 12-14 marzo 2008, Volume 1**,** 167-174.

Turnock, W.J., Fields, P.G., 2005. Winter climates and coldhardiness in terrestrial insects. European Journal of Entomology 102**,** 561-576.

Urbaneja, A., Mari, F.G., Tortosa, D., Navarro, C., Vanaclocha, P., Bargues, L., Castanera, P., 2006. Influence of ground predators on the survival of the mediterranean fruit fly pupae, Ceratitis capitata, in Spanish citrus orchards. Biocontrol 51**,** 611-626.

Vargas, R.I., Walsh, W.A., Jang, E.B., Armstrong, J.W., Kanehisa, D.T., 1996. Survival and development of immature stages of four Hawaiian fruit flies (Diptera: Tephritidae) reared at five constant temperatures. Annals of the Entomological Society of America 89**,** 64-69.

Vera, M., Leitão, F., 2008. Formigas (Hymenoptera, Formicidae) associadas a pomares de citrinos na região do Algarve. University of Lisbon.

Vera, M.T., Rodriguez, R., Segura, D.F., Cladera, J.L., Sutherst, R.W., 2002. Potential geographical distribution of the Mediterranean fruit fly, Ceratitis capitata (Diptera : Tephritidae), with emphasis on Argentina and Australia. Environmental Entomology 31**,** 1009-1022.

4. CHAPTER II: Evaluation of mass trapping equipment against *Ceratitis* spp. on the Island of La Réunion

INDEX

1 INTRODUCTION 97

2 MATERIAL AND METHODS 99

2.1 TRAPS TRIAL 100

2.2 ATTRACTANTS TRIAL 101

2.3 INSECTICIDES TRIAL 102

2.4 SYSTEMS TRIAL 103

3 RESULTS 104

3.1 TRAPS TRIAL 104

3.2 ATTRACTANTS TRIAL 108

3.3 INSECTICIDES TRIAL 111

3.4 SYSTEMS TRIAL 115

4 DISCUSSION 119

5 CONCLUSIONS 122

6 REFERENCES 122

1 INTRODUCTION

It is necessary to test the equipment used in mass trapping in areas where the technique is going to be used, because differences in the climatic conditions will impact on their effectiveness. One place where this methodology could be implemented is La Réunion Island (France). This tropical island comprises an area of 2,512 km^2 and is located in the Indian Ocean in the Southern hemisphere (Longitude 55º29' East and latitude 21º 53' South) (Duyck, 2005).

The first observations of *Ceratitis capitata* (Wiedemann) in La Réunion Island were made in 1939 (Etienne, 1982), the insect having probably been introduced at the beginning of the 20^{th} century (De Meyer, 2000). Because of its well-known polyphagous activity, medfly was found in nine botanic families (Etienne, 1982), some corresponding to important cultivated hosts, including arabica coffee (*Coffea arabica* Linnaeus), mango (*Magnifera indica* Linnaeus) and sour orange (*Citrus aurantium* Linnaeus) (White and Elson-Harris, 1992).

The *Ceratitis* genus comprises more than 90 species native to the Afro-tropical region (De Meyer, 2001), and a polyphagous congeneric species *Ceratitis rosa* Karsch, also known as the Natal fruit fly or *Pterandrus flavotibialis* Hering, which is a potentially serious pest (White and Elson-Harris, 1992). It belongs to the subgenus *Pterandrus* Bezzi, subfamily Dacinae, tribe Ceratitini and subtribe Ceratitina (White and Elson-Harris, 1992) (Quilici and Franck, 1999). This species was described in 1887 (De Meyer and Freidberg, 2005) and became established in La Réunion Island by 1955, through accidental introductions (Etienne, 1982). Nowadays it is found in a large part of the African continent, especially in the South, East, centre and Western areas (Commonwealth-Institute-of-Entomology, 1985) (De Meyer, 2001) and it has also been recorded in the United States (Weems and Fasulo, 2007). By 1900 it was recognized as a pest of orchard fruits (Weems and Fasulo, 2007) and it is currently listed as a major pest in commercial fruits (De Meyer, 2001).

C. rosa is the most important of *Ceratitis* spp. (*C. capitata* and the Mascarenes fruit fly, *Ceratitis catoirii* Guérin-Ménville,) in La Réunion Island because of its extensive distribution (Etienne, 1982), its ability to compete with and displace medfly (De Meyer, 2001) (Duyck et al., 2004), and its extensive host range of fifty six plants, the most important being mango, apricot (*Prunus armeniaca* Linnaeus) and peach (*P. persica* (Linnaeus)) (Quilici and Franck, 1999).

The attack of *C. rosa* is represented by early maturation and fruit fall. In the absence of treatment of the most susceptible hosts such as peach, damage by *C. rosa* can lead to complete yield loss (Quilici and Franck, 1999). The current control recommendations against these Tephritid in La Réunion are part of a system of integrated control, which has been used successfully with citrus fruits and mangos. The techniques used are male trapping, monitoring with the sexual attractant trimedlure (also used for *C. capitata*), poisoned bait, spraying using protein hydrolysate mixed with insecticide and cultural measures. The most common cultural measures are the destruction or burying of fallen fruit and the elimination of alternative host reservoirs close to the orchard. A chemical treatment is recommended when the number of flies registered per trap per week reaches 25 (Quilici and Franck, 1999).

Although originally it was not a target species, the unexpected findings of this study prompted the evaluation of a third species, *Bactrocera cucurbitae* (Coquillett), also known as the Melon fly, which belongs to the sub-family Dacinae and the tribe Dacini (Vayssières and Coubès, 1999). It was first identified in Réunion Island in 1972 (Vayssières and Coubès, 1999) and is currently considered to be one of the most harmful pests in cucurbit crops (Ryckewaert et al., 2010), where it has been known to induce total losses in untreated orchards (Ryckewaert et al., 2010). However, it is a polyphagous species, which attacks other hosts such as tomato (*Lycopersicum esculentum* Miller) and passion fruit (*Passiflora edulis* Sims) (Vayssières and Coubès, 1999).

Medfly has become invasive throughout the world, whereas *C. rosa* has not, perhaps because of differences in its morphological, physiological or behaviourally adaptive traits (Duyck et al., 2006). Nevertheless, some studies indicate that *C. rosa* might be more sensitive to low humidity and more tolerant of colder and wetter conditions than medfly. It has a lower larval development threshold than medfly, 3.1ºC compared with 10.2ºC (Duyck and Quilici, 2002) (Duyck et al., 2004) (Duyck et al., 2006), which suggests that it has a greater potential for establishment in temperate regions. Therefore, *C. rosa* could be a global threat to other areas including Girona because there is a high confidence in its predicted presence for the entire Spanish Mediterranean coast (De Meyer et al., 2008) and it would be useful to have equipment capable of detecting its first invasions.

A comparative study carried out in La Réunion Island with the trapping equipment already tested in field trials in Girona would be an important tool for the control of medfly and *C. rosa* in this island.

The insecticide currently used in mass trapping is dichlorvos or 2.2-dichlorovinyl dimethyl phosphate (DDVP). In the late fifties it was reported to prevent the escape of most flies once inside the trap and to kill some medfly individuals even before they entered the trap (Steiner, 1957). Recently it has been excluded from the European normative 91/414/CEE (EEC, 1991), although in 2010 it is still permitted for use under exceptional authorization. The trial of insecticides of this chapter belongs to a development study to find a suitable replacer to DDVP, and was carried out with the same active ingredients tested in Girona area (chapter III of this Ph.D.).

The aim of this field study was to evaluate the effectiveness of trapping equipment for *Ceratitis* spp. through comparative studies of trap types, attractants, insecticides and their different combinations (systems) in La Réunion Island.

2 MATERIAL AND METHODS

Four trials were carried out in commercial mandarin plots (*Citrus reticulata* Blanco) on La Réunion Island involving comparison of traps (T.), attractants (A.), insecticides (I.) and systems (S.) (Table 6). A. and I. trials were located in the same orchard, separated by 75 meters of citrus trees and in other plot placed few kilometres away T. and S. trials were conducted, separated by 100 meters. All four studies were performed within the locality of Petite-ile.

Table 6. Description of orchards from the comparative trials: trial identification letter, size, variety, plantation frame (distance among trees), height above sea level (altitude) and installation date.

TRIAL	SURFACE (ha)	MANDARIN VARIETY	PLANTATION FRAME (m)	ALTITUDE ABOVE SEA LEVEL (m)	INSTALLATION DATE
T.	0.50	'Zanzibar'	6 x 6	300 - 350	10/03/2009
A.	0.16 0.20	'Zanzibar' & 'Clementine'	5 x 5	350	6/03/2009
I.	0.14	'Zanzibar'	5 x 5	350	6/03/2009
S.	0.50	'Clementine'	6 x 6	350	4/03/2009

Three treatments were evaluated in each trial (Table 7). The experimental design used randomized blocks with four replicates (12 traps/trial) over a monitoring period lasting between 55 and 60 days. Traps were hung on citrus trees at a height of 1.5 m. Two clockwise rotations were made each week in order to diminish the possible effect of trap location, giving a total of 5 complete rotations. One cycle covers the period that a treatment needs to be located in the position where it was originally installed (a complete rotation).

In A., T. and S. trials, the number of dead males and females were recorded, while in I. trial, live and dead individuals were taken into account.

In each trial, the ratio of males/females was analyzed statistically to check if there were significant differences between treatments in a single cycle, through one-way ANOVA, using Tukey's studentized test, $P < 0.05$ (Enterprise Guide, SAS).

In each trial, the percentage of mean captures was pooled for each rotation cycle and then transposed (arcsine of the square root of the percentage of captures) and analyzed statistically through one-way ANOVA, using Tukey's studentized test, $P < 0.05$ (Enterprise Guide, SAS). In A., T. and S. trials, the analysis were carried out with the total captures, while in I. trial the analysis was carried out for live, dead and the total of individuals.

The closest weather station to the survey area was located at a height of 168 m which was 182 m lower than the plots in the trials. Temperature and rainfall was recorded throughout the study period.

Table 7. Characterization of the trapping equipment from the comparative trials.

TRIAL CODE	EQUIPMENT IDENTIFICATION	ATTRACTANT	TRAP	INSECTICIDE
T.	Maxi Tephri Easy	Ferag® CC D TM	Maxitrap® Tephri-trap® Easy-trap®	Ferag® ID TM
A.	Ferag BioLure U. BioLure 3C.	Ferag® CC D TM BioLure® Unipak BioLure® Med Fly	Maxitrap®	Ferag® ID TM
I.	DDVP A-C. D.	Ferag® CC D TM	Maxitrap®	Ferag® ID TM Alpha-cipermethrin Deltamethrin
S.	Ferag+Maxi+DDVP B.U.+Tephri+DDVP Cera Trap	Ferag® CC D TM BioLure® Unipak Cera Trap® lure	Maxitrap® Tephri-trap® Cera Trap®	Ferag® ID TM Ferag® ID TM None

2.1 TRAPS TRIAL

Traps used in T. trial were Maxitrap® trap (Probodelt, S.L., Amposta, Spain) Tephri-trap® (Sorygar, S.L., Madrid, Spain) and Easy-trap® (Sorygar, S.L.) (Figure 18).

Maxitrap® trap is a plastic device composed of a yellow base and a transparent lid with a hanger. The bottom part has three holes, 2.5 cm in diameter each one with a transparent tube which helps to avoid the exit of flies, and another hole in the base (Lucas and Hermosilla, 2008d).

Tephri-trap® is a yellow truncated cone 11 cm deep, fitted with an opaque lid (3.5 cm deep). The bottom part has four fly entry holes, 2.1 cm in diameter, which are positioned at 90º to each other. As in the previous trap model, there is another entrance hole in the base (Broughton and De Lima, 2002).

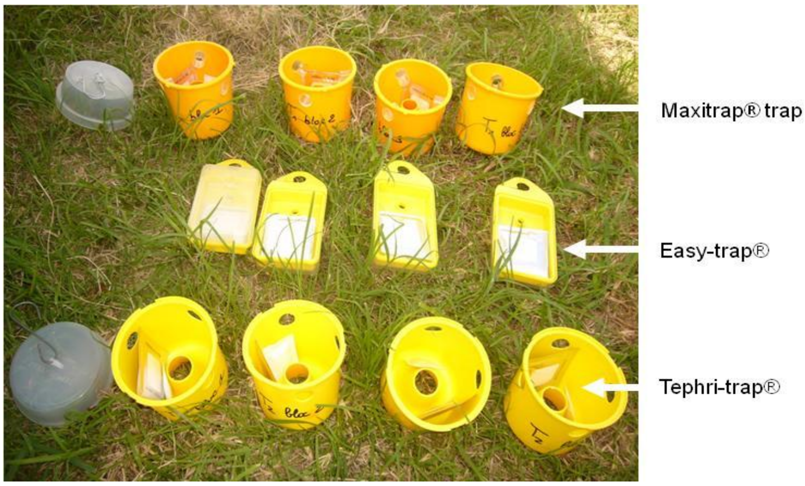


Figure 18. Different trap models used in the T. trial, all filled with the same attractant and the same insecticide. Photo: E. Peñarrubia.

Easy-trap® is a plastic trap composed of two rectangular parts, one yellow and other transparent linked by the central part. Both rectangles have a hole and one of the parts contain a hanger (Lucas and Hermosilla, 2005).

In this trial the same lure and insecticide were used in all cases, Ferag® CC D TM (SEDQ S.L., Barcelona, Spain, described in 2.2) and Ferag® ID TM (SEDQ S.L., described in 2.3), respectively.

2.2 ATTRACTANTS TRIAL

Lures tested were Ferag® CC D TM, BioLure® Unipak and BioLure® Med Fly (Suterra España Biocontrol S.L., Cerdanyola del Vallès, Spain) (Figure 19).

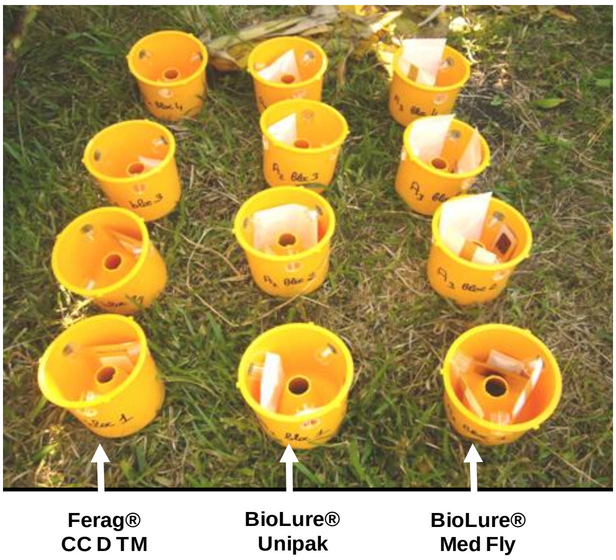


Figure 19. Different attractants used in the A. trial, all installed in the same trap model with the same insecticide. Photo: E. Peñarrubia.

The commercial formulation Ferag® CC D TM is a food-based synthetic lure containing ammonium acetate (7.8 g), diaminoalkane (0.03 g) and trimethylamine (2.5 g) formulated in a unique and compact dispenser in the shape of a sachet (Lucas and Hermosilla, 2008d).

BioLure® Unipak is a lure composed of ammonium acetate (6.19 g), putrescine (1.4-diaminobutane) (0.05 g) and trimethylamine hydrochloride (2.81 g) in a unique and compact dispenser (SUTERRA, 2007) (Lucas and Hermosilla, 2008d). This attractant was formulated in order to improve the handling of BioLure® Med Fly, which had previously contained several dispensers per trap that were time-consuming to set up.

BioLure® Med Fly is a three component food-bait composed of the same set of chemical substances as the previous lure but in different amounts in three separated dispensers, one for each compound: ammonium acetate (3,92 g), putrescine (1.4-diaminobutane) (0.05 g) and trimethylamine hydrochloride (1.69 g) (SUTERRA, 2007).

According to the manufacturers, the longevity of all lures is 120 days, except for BioLure® Med Fly that lasts 45 days. The attractants in this model were therefore replaced once, in order to cover the full 60 days of the trial.

As in the previous trial, all treatments were administered in the same trap, Maxitrap® (described in 2.1), using the same insecticide, Ferag® ID TM (DDVP).

2.3 INSECTICIDES TRIAL

The insecticides tested were Ferag® ID TM, alpha-cipermethrin and deltamethrin, all manufactured by SDEQ, S.L (Figure 20).

Figure 20. Different insecticides used in the I. trial. DDVP was installed inside the trap and alpha-cipermethrin and deltamethrin in the lid. All treatments were installed in the same model of trap and used the same attractants. Photo: E. Peñarrubia.

Ferag® ID TM consisted of 320 mg of dichlorvos or 2.2-dichlorovinyl dimethyl phosphate (DDVP) placed in cellulose dispensers that allowed a slow liberation of the product (2.2 mg/day and dispenser). According to the manufacturer, its longevity is 120 days (Lucas and Hermosilla, 2008d).

Alpha-cipermethrin 15 mg and deltamethrin 15 mg were administered by movement impregnation in a support linked to the base of the lid.

In I. trial Maxitrap® and Ferag® CC D TM were used in all treatments.

2.4 SYSTEMS TRIAL

The commercial complete equipments or systems tested were varying combinations of the previous traps and attractants (Figure 21):

1. Maxitrap® baited with Ferag® CC D TM and the insecticide Ferag® ID TM (Ferag+Maxi+DDVP).
2. Tephri-trap® baited with BioLure® Unipak and the same insecticide (BioLure U.+Tephri+DDVP).
3. Cera Trap® (Bioibérica S.A., Barcelona, Spain) baited only with Cera Trap® lure (Bioibérica S.A.).

Figure 21. Different systems used in the S. trial. Photo: E. Peñarrubia.

Cera Trap® is similar to Maxitrap® trap but 3 cm taller (BIOIBÉRICA, 2006) and its lure was the only liquid attractant used (between 250 and 350 ml per trap), which avoided the necessity of adding an insecticide because flies died by drowning. It is composed of hydrolyzed proteins 95% plus additives 5% (BIOIBÉRICA, 2006) (Lucas and Hermosilla, 2008b). This causes the emission of amines and organic acid compounds which are highly attractive to young or immature female fruit flies (Piñol, 2009).

3 RESULTS

The total number of individuals captured over the entire trial period of all four trials for all treatments was 49,883 adults.

This study was performed among mandarin trees, which are known hosts for the two target species, *C. capitata* and *C. rosa* (Quilici and Jeuffrault, 2001). However, in addition to these species six more fruit flies species were identified: *B. cucurbitae*, *Bactrocera zonata* (Saunders) (Peach fruit fly), *C. catoirii*, *Dacus ciliatus* Loew (The Ethiopian cucurbit fly), *D. demmerezi* (Bezzi) (Indian Ocean cucurbit fly) and *Neoceratitis cyanescens* (Bezzi) (Tomato fly) (Figure 22). The most captured species in all four trials were *C. rosa* followed by *B. cucurbitae* and by *C. capitata*, while the proportions of the other species varied between 0.31 and 1.03% in T. trial, 0.11 and 0.64% in A. trial, 0.05 and 0.62% in I. trial and 0.61 and 2.47% in S. trial.

The mean temperature throughout the trials was 25.3ºC, which coincides with the optimal temperature for the survival of the target species. Rainfall was also recorded throughout the study period.

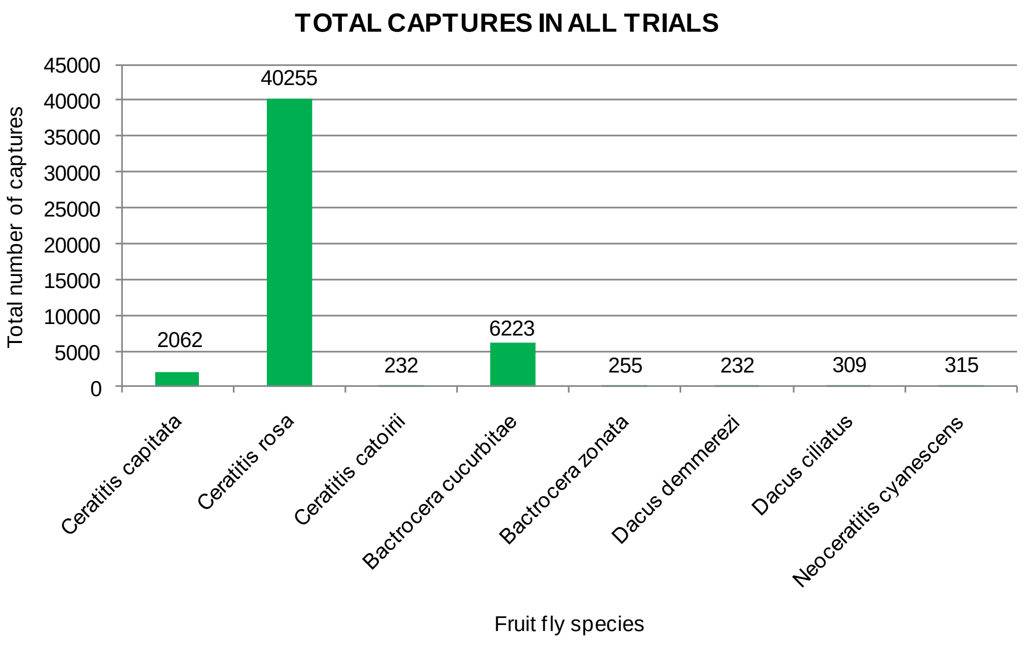


Figure 22. Total number of captures of each species from all comparative trials.

3.1 TRAPS TRIAL

The percentages of the most captured fruit fly species in the traps trial were 52.69% (*C. rosa*), 33.30% (*B. cucurbitae*) and 11.37% (*C. capitata*).

Rainfall registered during the five cycles of the T. trial was 23 mm, 15 mm, 419 mm, 150 mm and 134 mm.

No significant differences were found in the proportion of males to females of the three most captured species in any of the three treatments used (Table 8).

Table 8. Average value of the ratio males/females of *C. rosa*, *B. cucurbitae and C. capitata* captured in each treatment over the time. Values followed by the same letter in the same column are not significantly different (Tukey's studentized test, P < 0.05) n = 4.

TRAP	SPECIES	CYCLE									
		1		2		3		4		5	
Maxitrap® trap	*C. rosa*	1.15	a	0.87	a	0.65	a	0.80	a	0.33	a
Tephri-trap®		1.65	a	1.42	a	0.78	a	0.78	a	0.45	a
Easy-trap®		0.94	a	0.88	a	0.73	a	1.21	a	0.37	a
		p=0.6816		*p=0.0565*		*p=0.8200*		*p=0.1730*		*p=0.4919*	
Maxitrap® trap	*B. cucurbitae*	1.78	a	1.19	a	0.81	a	0.96	a	1.07	a
Tephri-trap®		0.62	a	1.21	a	1.07	a	0.95	a	0.90	a
Easy-trap®		0.17	a	1.39	a	1.27	a	0.94	a	1.40	a
		p=0.3250		*p=0.9676*		*p=0.4710*		*p=0.9985*		*p=0.6000*	
Maxitrap® trap	*C. capitata*	0.25	a	0.58	a	0.44	a	0.99	a	1.00	a
Tephri-trap®		0.00	a	0.25	a	0.53	a	0.76	a	0.92	a
Easy-trap®		0.00	a	0.00	a	0.29	a	0.64	a	1.31	a
		p=0.4053		*p=0.4484*		*p=0.7327*		*p=0.2509*		*p=0.1890*	

Statistical analysis of the different responses of traps is illustrated graphically on the mean percentage bar chart. Easy-trap® did not exceed 21% of captures of any species in any cycle. Analysis of data on *C. rosa*, showed that in all but the 3rd cycle, Maxitrap® and Tephri-trap® produced statistically similar results, while in all cases Easy-trap® had the lowest proportion of captures, with significant differences (Figure 23). As with the previous species, captures of *B. cucurbitae* showed significant differences between the cluster formed by Maxitrap® and Tephri-trap®, both of which achieved a higher proportion of captures than Easy-trap® throughout the study period (Figure 23). In all but one cycle Tephri-trap® registered a similar proportion of medfly captures than Maxitrap®. In the two first cycles no significant differences between treatments were found, but over the three last ones Easy-trap® captured significantly fewer proportion of medfly than Tephri-trap® (Figure 23).

The graph showing the accumulated sum of captures of *C. rosa* and *B. cucurbitae* illustrates the already mentioned difference between Easy-trap® and the other two models (Maxitrap® and Tephri-trap®), being these which achieved a higher number of captures (Figure 24). The lowest numbers of medfly captures in the trial were found in the two first cycles, when 3 and 29 flies were registered, respectively. In these cycles only 0 and 1 flies were captured using Easy-trap® (Figure 24). Although at the beginning of the trial there were similarities between the numbers of medfly captures in all three traps, as time went by, the accumulated catch differed between treatments, being much lower for all three species in the Easy-trap (Figure 24).

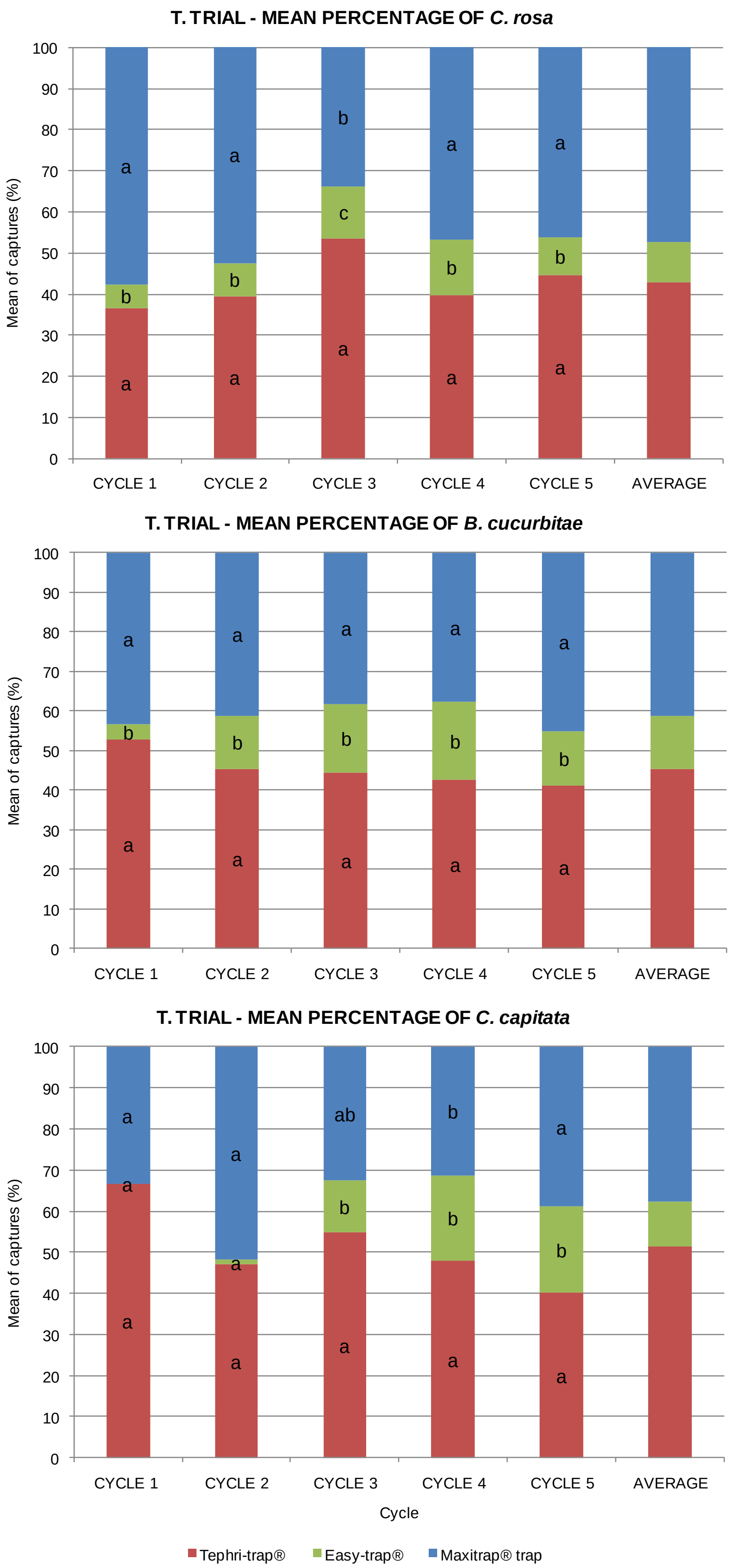


Figure 23. Mean percentage of captures from each species found inside each trap model over the study period. Values followed by the same letter in the same column are not significantly different (Tukey's studentized test, $P < 0.05$) n = 4.

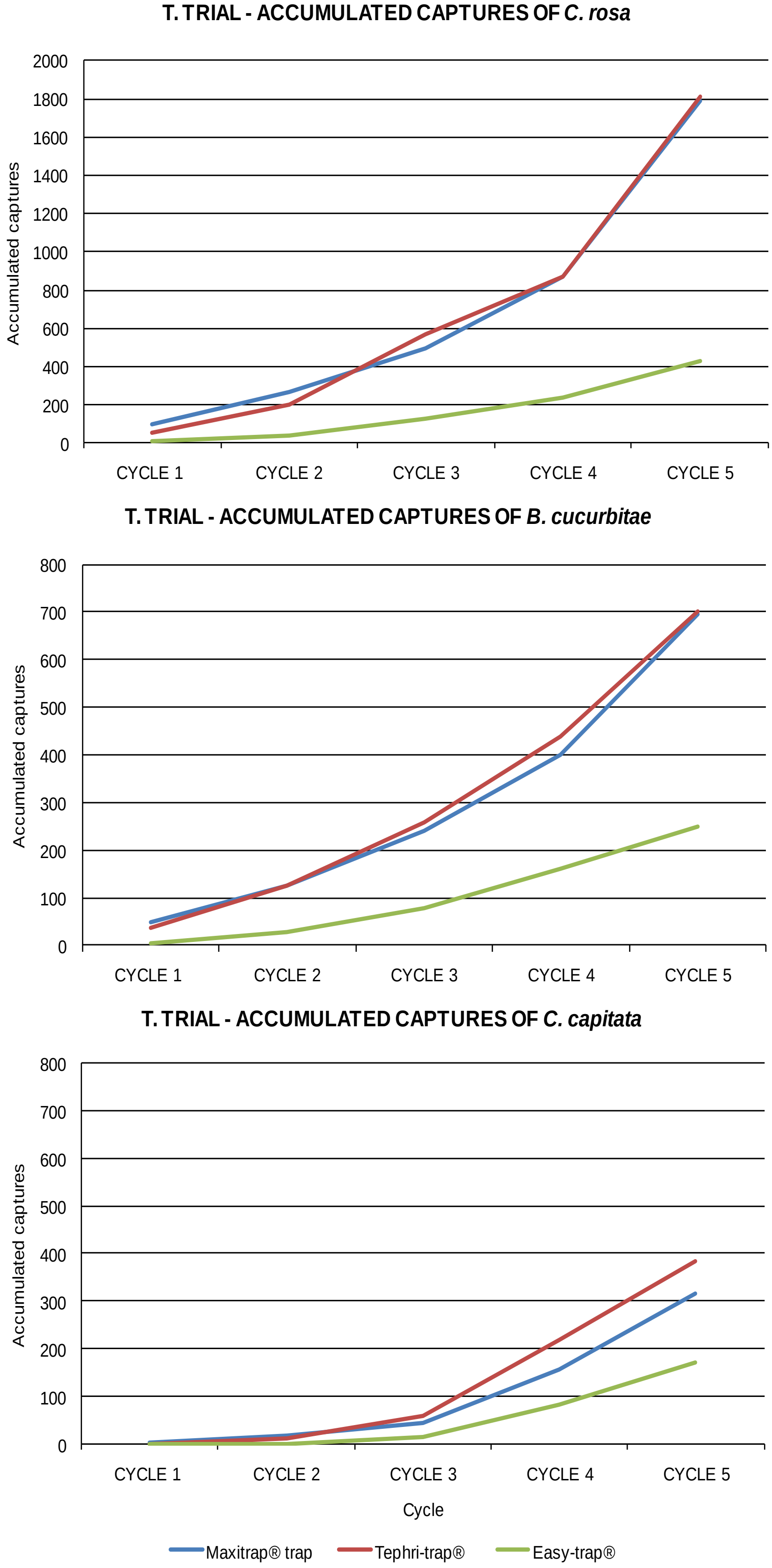


Figure 24. Accumulated number of captures of each species found inside the different traps over the studied period.

3.2 ATTRACTANTS TRIAL

The percentage of the most captured fruit fly species in the lures trial were 92.40% for *C. rosa,* 4.42% for *B. cucurbitae* and 1.08% for *C. capitata*.

Rainfall registered in each cycle of the A. trial was 15.5 mm, 27.5 mm, 407 mm, 72.5 mm and 227.5 mm.

No significant differences were found between the attractants evaluated in respect of the ratio of males to females of *C. rosa*, *B. cucurbitae* and medfly captured (Table 9).

Table 9. Average of males/females of *C. rosa*, *B. cucurbitae* and *C. capitata* for each treatment over the study period. Values followed by the same letter in the same column are not significantly different (Tukey's studentized test, P < 0.05) n = 4.

ATRACTANT	SPECIES	CYCLE									
		1		2		3		4		5	
Ferag® CC D TM	*C. rosa*	0.68	a	0.61	a	0.62	a	0.55	a	0.56	a
BioLure® Unipak		0.53	a	0.55	a	0.61	a	0.54	a	0.54	a
BioLure® Med Fly		0.45	a	0.53	a	0.52	a	0.59	a	0.60	a
		p=0.2938		*p=0.7818*		*p=0.5877*		*p=0.7230*		*p=0.4971*	
Ferag® CC D TM	*B. cucurbitae*	0.41	a	0.19	a	1.87	a	0.61	a	1.36	a
BioLure® Unipak		0.52	a	0.83	a	0.25	a	1.54	a	1.36	a
BioLure® Med Fly		1.81	a	1.06	a	0.55	a	1.15	a	1.78	a
		p=0.1387		*p=0.3202*		*p=0.3801*		*p=0.6478*		*p=0.5761*	
Ferag® CC D TM	*C. capitata*	0.00	a	0.25	a	0.00	a	1.21	a	0.36	a
BioLure® Unipak		0.25	a	0.27	a	0.25	a	0.71	a	0.54	a
BioLure® Med Fly		0.13	a	0.25	a	0.21	a	0.60	a	0.69	a
		p=0.5694		*p=0.9975*		*p=0.5273*		*p=0.6229*		*p=0.7397*	

Significantly higher proportion of captures of *C. rosa* were found in three out of five cycles when using BioLure® Med Fly (three dispensers) and BioLure® Unipak. Nevertheless, Ferag® CC D TM did not differ from BioLure® Unipak in the ratio mentioned above (three out of five cycles) (Figure 25). Although none of the attractants used were specific of *B. cucurbitae*, BioLure® Unipak was significantly different from Ferag® CC D TM and from BioLure® Med Fly in the last two cycles. Despite the similarity in the accumulated number of captures of *B. cucurbitae* using Ferag® CC D TM and BioLure® Med Fly over the study period (Figure 26), in the first and last cycle there were statistical differences between them in the mean percentage of captures recorded (Figure 25). Over the five cycles of the A. trial, the number of medfly adults found in each treatment varied from 2 to 30 and the statistical analysis showed no differences between treatments in any cycle (Figure 25).

The different levels of captures of the species could be due to the population levels or to differences in the effectiveness of the equipment (Figure 26).

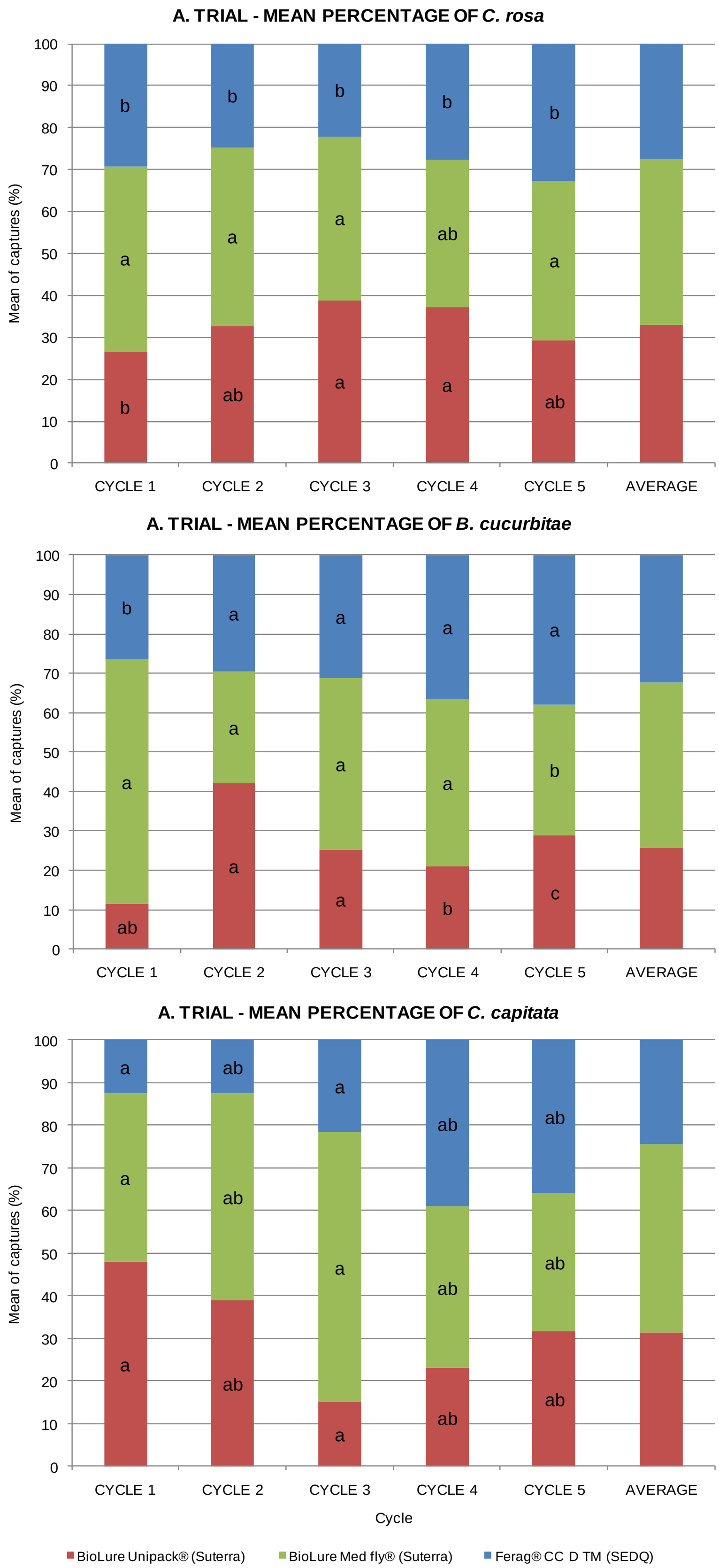


Figure 25. Mean of the percentage of captures from each species found using the three attractants over the study period. Values followed by the same letter in the same column are not significantly different (Tukey's studentized test, $P < 0.05$) n = 4.

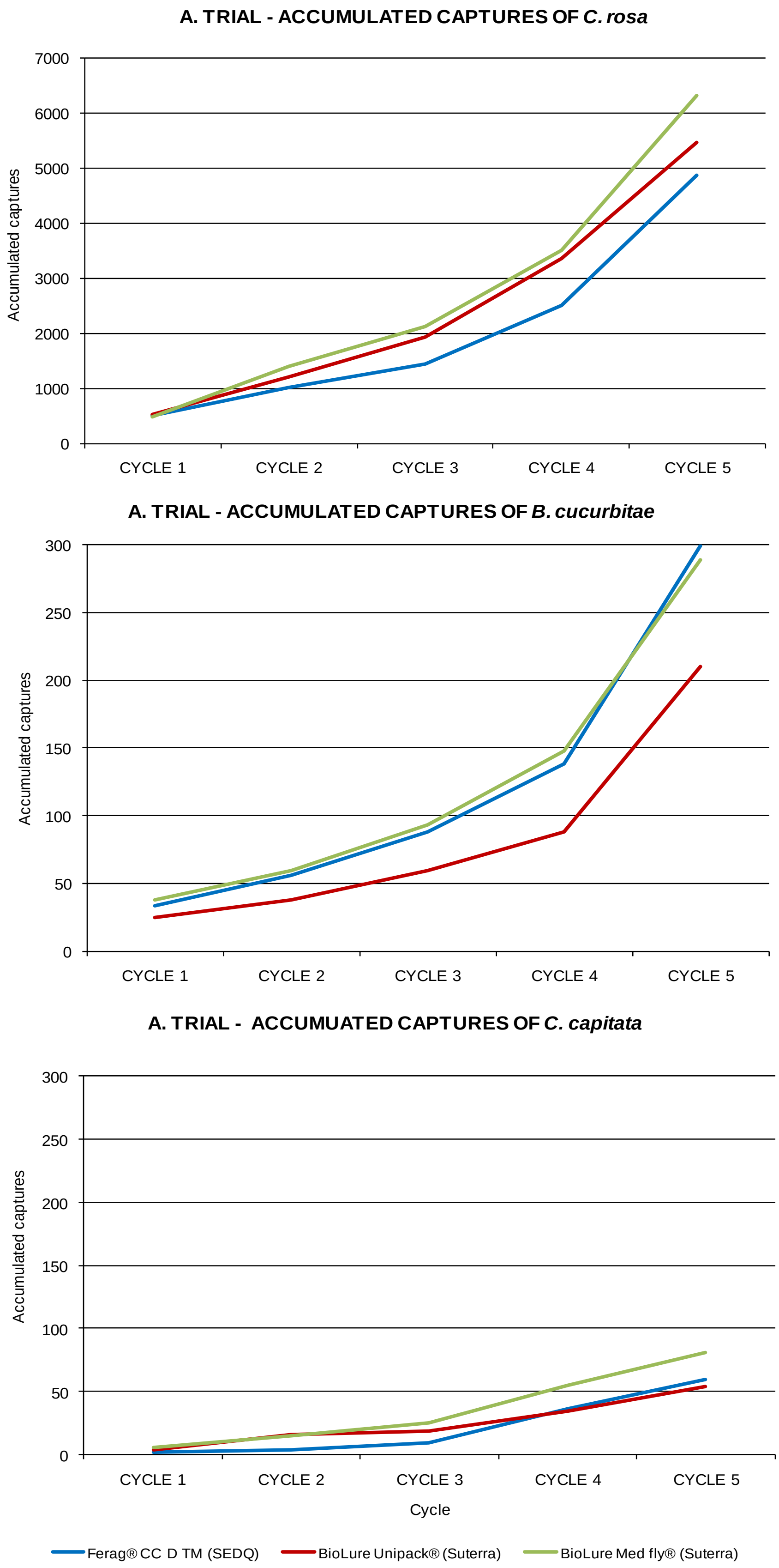


Figure 26. Accumulated number of captures of each species found using the different attractants over the studied period.

3.3 INSECTICIDES TRIAL

The percentage of the most captured fruit fly species in the insecticide trial were 96.04% for *C. rosa*, 2.53% for *B. cucurbitae* and 0.36% for *C. capitata*.

Rainfall registered in each cycle of the I. trial was the same than in A. Trial.

No live adult medfly was recorded in the traps used in the I. trial, and only 49 dead ones were counted. Despite the low level of captures of *C. capitata*, similar numbers of dead flies were observed for all treatments. Owing to the extremely low number of live *B. cucurbitae* flies (seven individuals) neither a table nor a chart has been drawn.

The percentage of live flies found inside the traps must be as low as possible. Alpha-cipermethrin reached significant higher live flies of *C. rosa* and DDVP registered fewer surviving adults, followed by deltamethrin, although it was significant only in the second cycle (Figure 27).

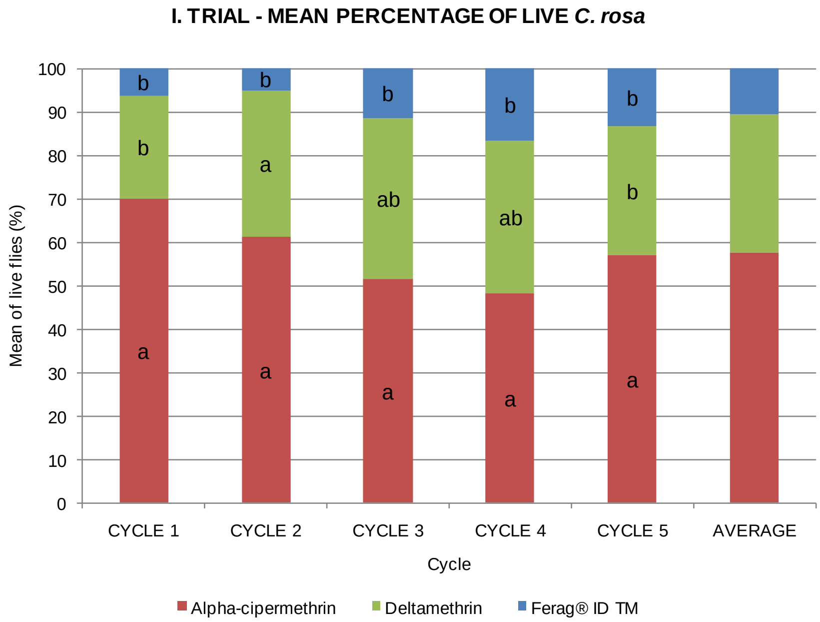


Figure 27. Mean of the percentage of *C. rosa* captures found live using the three insecticides over the study period. Values followed by the same letter in the same column are not significantly different (Tukey's studentized test, P < 0.05) n = 4.

Dead flies of *C. rosa* using deltamethrin did not differ significantly with DDVP in four cycles, being followed by alpha-cipermethrin (Figure 28).

Dead flies of *B. cucurbitae* followed a similar pattern to those from *C. rosa*, with no significant differences when using alpha-cipermethrin (the insecticide with lowest captures) and the other two treatments. The only exception was in the fifth cycle, in which this insecticide produced significantly fewer captures than DDVP (Figure 28).

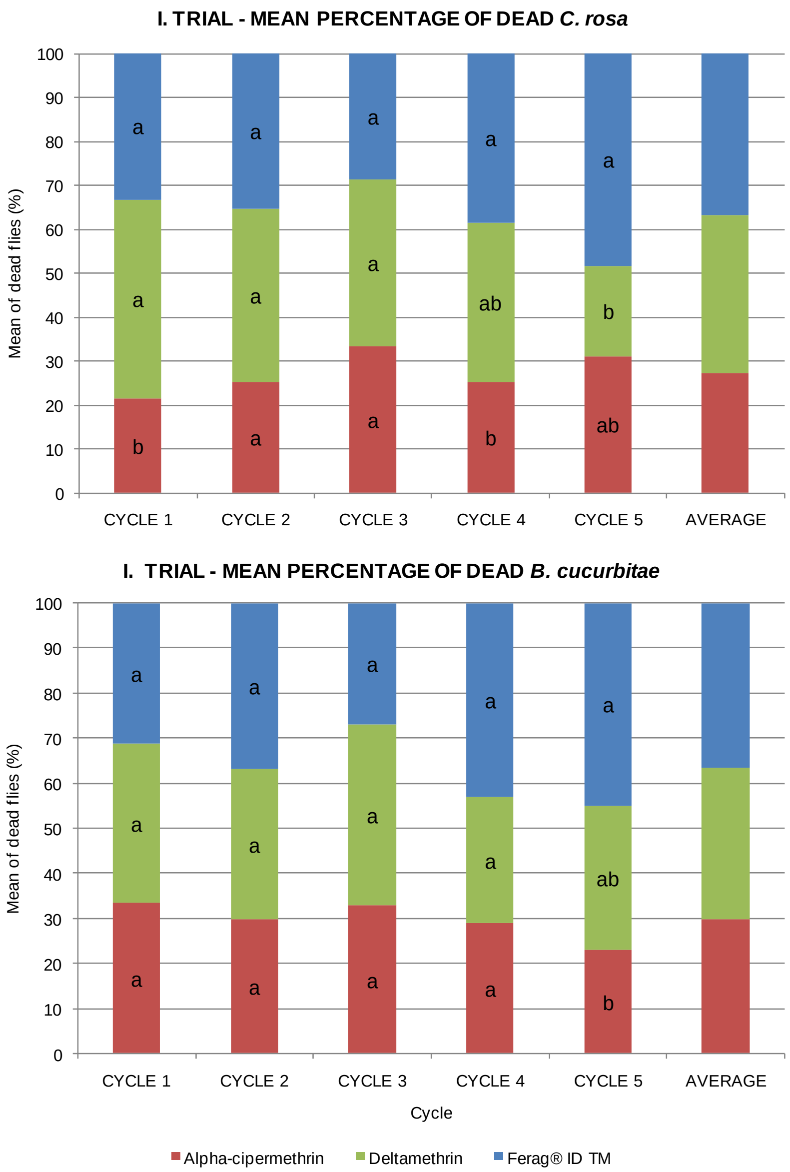


Figure 28. Mean percentage of *C. rosa* and *B. cucurbitae* found dead using the three insecticides over the studied period. Values followed by the same letter in the same column are not significantly different (Tukey's studentized test, $P < 0.05$) n = 4.

Due to the low level of live captures of *C. rosa*, analysis of total captures (live and dead flies) produced similar results to the analysis of only dead ones. The only difference found between the analysis of dead flies (Figure 28) and the analysis of total flies (Figure 29) took place in the first cycle. In this cycle and for the total flies, DDVP did not differed from the other two treatments.

The difference between accumulated captures of live *C. rosa* among the three treatments was observed in the first cycle and this difference increased over the time. Alpha-cipermethrin was the insecticide which obtained higher catches. This treatment was followed by deltamethrin and at a lower level by DDVP, which achieved a maximum of only 32 live individuals (Figure 30).

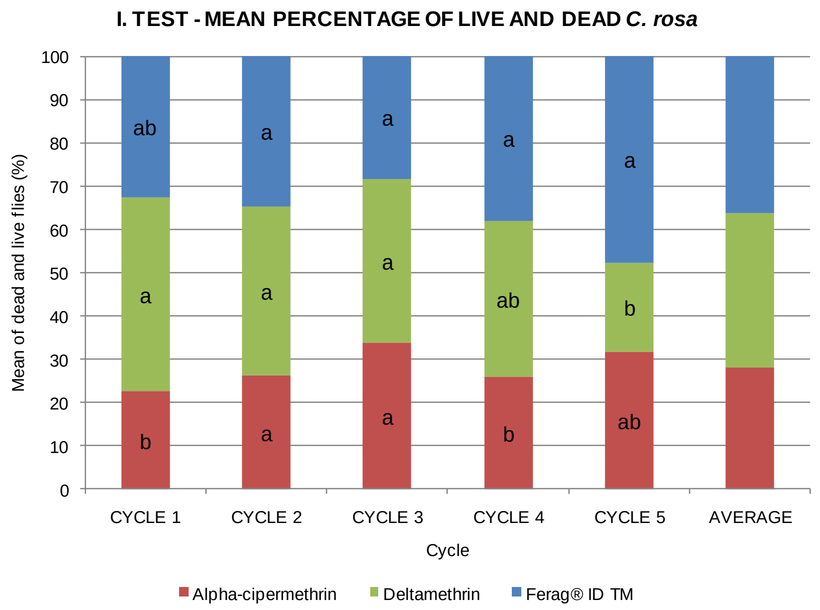


Figure 29. Mean percentage of *C. rosa* found live and dead using the three insecticides over the studied period. Values followed by the same letter in the same column are not significantly different (Tukey's studentized test, $P < 0.05$) $n = 4$.

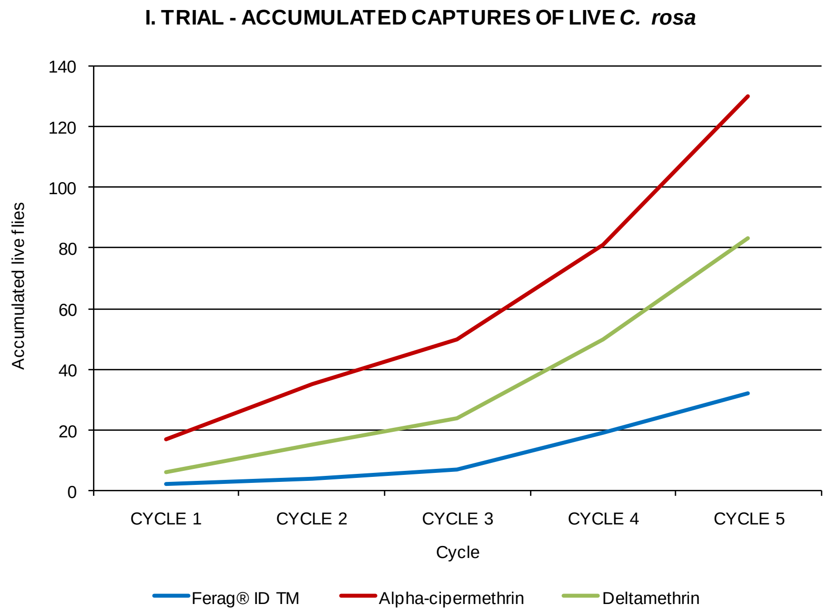


Figure 30. Accumulated number of live *C. rosa* captured with each treatment over cycles.

In the last part of the study the accumulated dead captures of *C. rosa* using DDVP registered a higher number of dead flies than the other two treatments (5,147 adults) (Figure 31).

The patterns of accumulated captures of dead *B. cucurbitae* were similar to those for dead *C. rosa*, but the maximum level of dead flies recorded was thirty nine times higher for *B. cucurbitae* when using DDVP or deltamethrin and thirty six times higher when using alpha-cipermethrin (Figure 31).

As it has been cited above, due to the low level of live captures of *C. rosa*, the accumulated chart of total captures (live and dead) was very similar to the chart of dead ones (Figure 32).

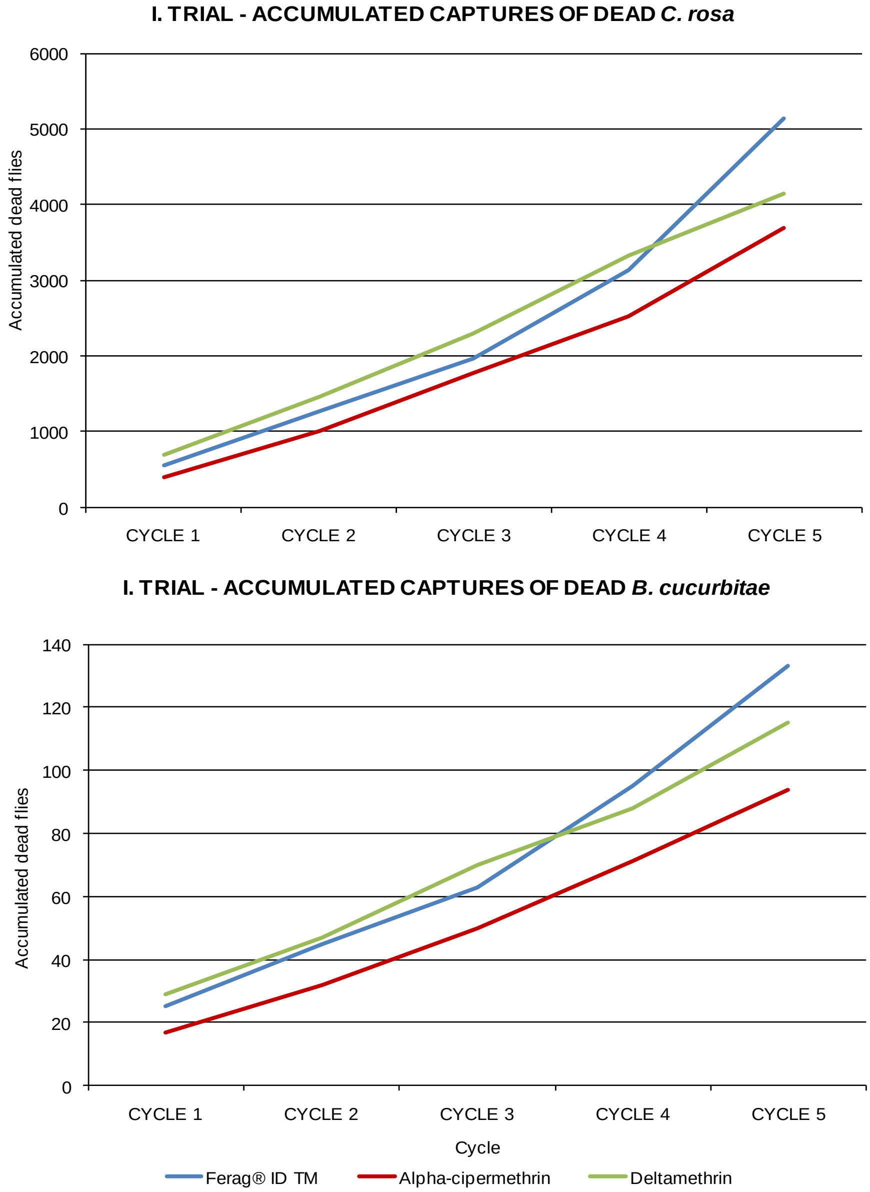


Figure 31. Accumulated number of dead *C. rosa* and *B. cucurbitae* captured with each treatment over cycles.

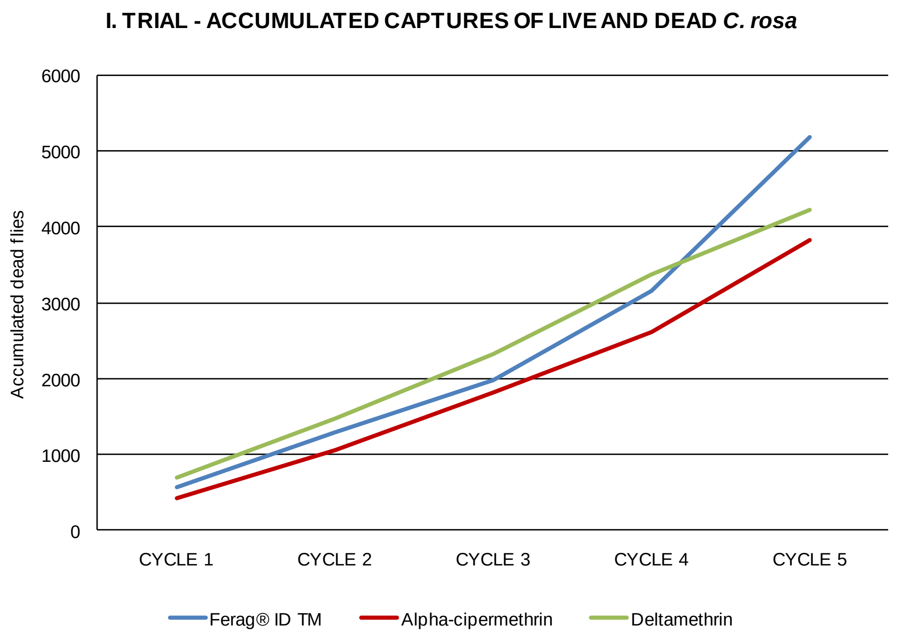


Figure 32. Accumulated number of live and dead *C. rosa* captured with each treatment over cycles.

3.4 SYSTEMS TRIAL

The percentages of the most captured fruit fly species in the systems trial were 60.71% for *C. rosa*, 24.28% for *B. cucurbitae* and 9.11% for *C. capitata*.

Rainfall registered in each cycle of S. trial was 13 mm, 34 mm, 5.5 mm, 425 mm and 143 mm, respectively.

In the present trial the ratio of male to female of *B. cucurbitae* showed significant differences in the first and the last cycle. The systems with higher ratio were Ferag+Maxi+DDVP and B.U.+Tephri+DDVP in the first cycle and B.U.+Tephri+DDVP in the last one (Table 10).

Systems baited with solid lures (Ferag+Maxi+DDVP and B.U.+Tephri+DDVP) did not differ statistically in their captures of *C. rosa*, but both obtained better results than the Cera Trap system. Comparing B.U.+Tephri+DDVP with Cera Trap, the former system obtained significantly higher captures in four out of five cycles. However, comparing Ferag+Maxi+DDVP with Cera Trap, there were no differences in four out of five periods (Figure 33).

Similar results were obtained for *B. cucurbitae* in all three systems over the study period. The trapping equipment composed of B.U.+Tephri+DDVP showed the lowest accumulated captures of this species (Figure 34), but only in the beginning of the trial the proportion of captures using it were significantly lower than the other treatments (Figure 33).

Table 10. Average ratio of males to females of *C. rosa*, *B. cucurbitae* and *C. capitata* for each treatment over the study period. Values followed by the same letter in the same column are not significantly different (Tukey's studentized test, P < 0.05) n = 4.

SYSTEM	SPECIES	CYCLE									
		1		2		3		4		5	
Ferag+Maxi+DDVP	*C. rosa*	0.71	*a*	0.46	*a*	0.37	*a*	0.27	*a*	0.39	*a*
B.U.+Tephri+DDVP		0.54	*a*	0.45	*a*	0.36	*a*	0.31	*a*	0.49	*a*
Cera Trap		0.58	*a*	0.43	*a*	0.40	*a*	0.37	*a*	0.35	*a*
		p=0.4742		*p=0.9465*		*p=0.8544*		*p=0.2220*		*p=0.1683*	
Ferag+Maxi+DDVP	*B. cucurbitae*	1.71	*a*	1.31	*a*	0.82	*a*	0.66	*a*	0.33	*b*
B.U.+Tephri+DDVP		1.39	*ab*	0.70	*a*	0.68	*a*	0.60	*a*	0.51	*a*
Cera Trap		0.87	*b*	0.73	*a*	0.56	*a*	0.59	*a*	0.33	*b*
		p=0.0422		*p=0.2647*		*p=0.6689*		*p=0.8967*		*p=0.0123*	
Ferag+Maxi+DDVP	*C. capitata*	1.00	*a*	2.05	*a*	0.13	*a*	0.93	*a*	1.06	*a*
B.U.+Tephri+DDVP		0.45	*a*	2.17	*a*	0.73	*a*	0.99	*a*	1.05	*a*
Cera Trap		0.69	*a*	0.54	*a*	0.42	*a*	1.76	*a*	0.95	*a*
		p=0.6160		*p=0.6328*		*p=0.3642*		*p=0.4241*		*p=0.9310*	

Despite that the proportion of medfly captures registered in Cera Trap was smaller than the other systems over all the studied period, significant differences were found in only three cycles. There were no differences between Ferag+Maxi+DDVP and B.U.+Tephri+DDVP in the proportion of captures, except in the third cycle, when the second treatment produced 1.7 times as many captures (Figure 33).

For the two species of the genus *Ceratitis*, Cera Trap performed less effectively than the other two systems. The proportion of captures of both species using this system did not exceed the 30% in any of the five cycles (Figure 33).

The average mean percentage of *B. cucurbitae* captures were similar across the three systems and B.U.+Tephri+DDVP captured the lowest proportion, although it differed statistically from the other two only in the first cycle (Figure 33).

In S. trial the sum of accumulated captures across the three systems varied with the species, i.e. there were 6,333 captures of *C. rosa*, 2,533 of *B. cucurbitae* and 950 of medfly (Figure 34).

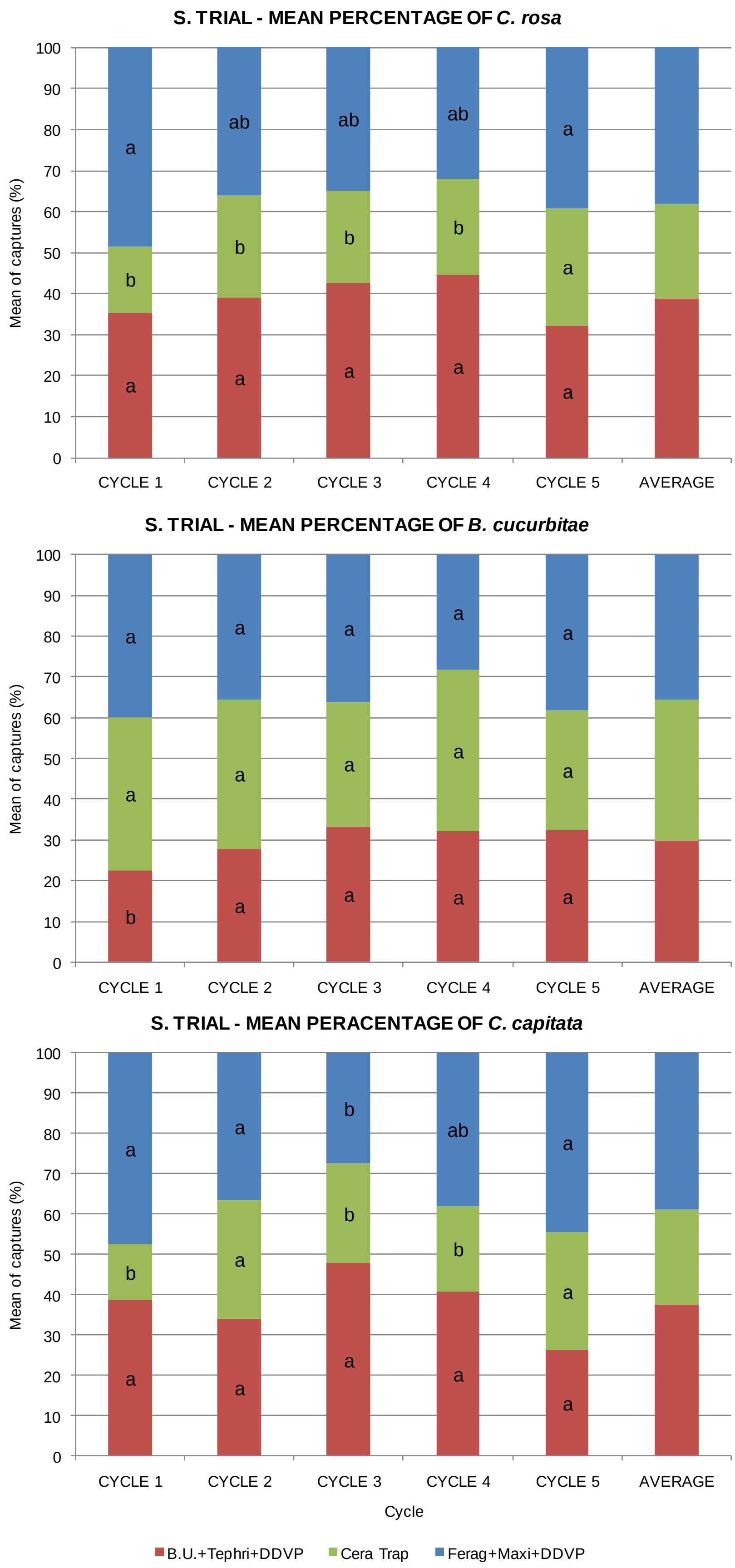


Figure 33. Mean percentage of captures from each species found using the three systems over the study period. Values followed by the same letter in the same column are not significantly different (Tukey's studentized test, $P < 0.05$) n = 4.

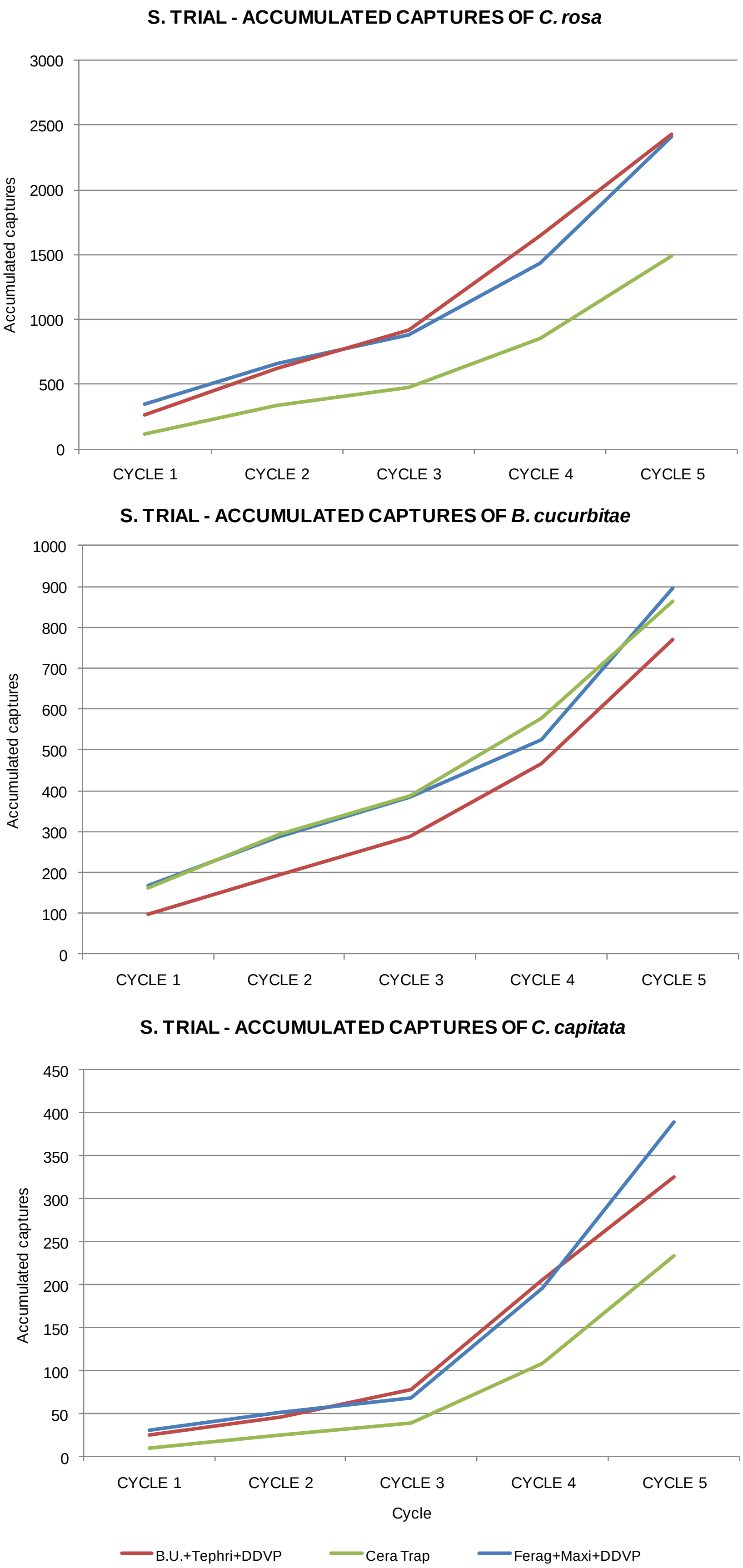


Figure 34. Accumulated number of captures of each species found using different systems over the time.

4 DISCUSSION

C. rosa was confirmed as the dominant species at heights between 100 m and 1,100 m (Quilici et al., 2005) (Duyck et al., 2006), though the plots from the study were at 300 to 350 m. Compared with the other Tephritid species, its proportion is higher than 90% in mangoes and 70 - 80% in citrus fruits (Etienne, 1982). The difference found in the proportions of each species could also be due to the different levels of efficacy of the trapping equipment used for each one.

On Réunion Island, the spatial distribution and density of populations of *B. cucurbitae* are particularly high from December to March (Ryckewaert et al., 2010). The present work took place between March and May but the captures level was sufficiently elevated for data analysis, even though originally, the aim of this study was to evaluate two species from *Ceratitis* genus.

The results found in the present work could be influenced by plot location, altitude and the fruit varieties planted. However, traps were installed in two orchards situated between 300 and 350 m above sea level and the same varieties were evaluated in both plots, 'Zanzibar' and 'Clementine'.

In respect of climatic factors, it was anticipated that high levels of rainfall would have a negative effect on the number of captures recorded, although there would be an increase a few days later (Christenson and Foote, 1960) (Cayol and Causse, 1993) (Appiah et al., 2009). This was corroborated in all four trials, where the percentage of captures gradually reduced during the third cycle in T., A. and I. trials and during the fourth cycle in S. trial coinciding with the periods with maximum rainfall registered. Nevertheless in the following cycle total captures of the three species across all treatments rose.

The ratio male to female for all three species was analyzed in each trial and similar results were obtained for the different treatments, as it has been found by other authors (Lucas and Hermosilla, 2008c). The only significant differences of this ratio were found with data of *B. cucurbitae* in two cycles of the S. trial.

Results obtained in T. trial were backed up by other studies, which showed that Maxitrap® performed more effectively against medfly than Easy-trap® in citrus groves (Lucas and Hermosilla, 2006) and nectarine orchards in Murcia, Spain (Lucas et al., 2006). However, several studies have published very different results regarding medfly, which indicated that Tephri-trap® and Easy-trap® did not differ significantly when tested using an attractant composed of the same lure in a fig orchard in Alicante, Spain (Vinaches et al., 2005) and in a citrus grove in Murcia (Lucas and Hermosilla, 2005). Nor differences were found when they were tested with an attractant containing the same lure in mango groves in Malaga, Spain (Ros et al., 2005b). A further study carried out in citrus orchards in Valencia found no differences between Tephri-trap® and Easy-trap® which obtained significant fewer adults of medfly than Maxitrap® (Navarro-Llopis et al., 2008).

The number and size of the holes in a trap may be key factors in its effectiveness. One possible reason for the low efficacy of the Easy-trap in the present study could be the absence of an aperture in its base, a factor which has been reported to be an important feature in trap design (Heath et al., 1996).

The effectiveness of the three-component lure has been shown to be strong when compared with other female attractants (Ros et al., 1997a) (Epsky et al., 1999) (Katsoyannos et al., 1999) (Miranda et al., 2001) (Broughton and De Lima, 2002). Therefore three lures were tested in A. trial, each composed of three components. BioLure® Med Fly obtained better results than Ferag® CC D TM for *C. rosa*, confirming the results of a trial conducted in citrus orchards in Murcia against medfly at high captures level (Lucas and Hermosilla, 2006). Although BioLure® Med Fly and Ferag® CC D TM were not specific attractants for *B. cucurbitae*, both obtained similar results at low captures level of this species, results that coincide with the study at low level of captures of medfly (Lucas and Hermosilla, 2006). No significant differences were found between these two attractants in another citrus study conducted in Valencia with low captures level of medfly (lower than 4 flies/trap/day) (Navarro-Llopis et al., 2008).

The similarity in the effectiveness of BioLure® Unipak and Ferag® CC D TM observed in the present work was also found in studies of medfly using Maxitrap® traps, such as that carried out in peach and citrus groves on Ibiza Island (Alonso-Muñoz and García-Marí, 2007) and the one conducted in a citrus orchard in Murcia, also using Maxitrap® (Lucas and Hermosilla, 2008d). However, very different results from those of the present study were obtained in a study were BioLure® Unipak recorded significantly higher captures than Ferag® CC D TM (Navarro-Llopis et al., 2008).

Variability in effectiveness of different attractants could be explained by variations in the diffusion of ammonium acetate and trimethylamine. This phenomenon was observed in a comparative study performed in 2005, where the largest trimethylamine emission was for BioLure® Unipak and the smallest for Ferag® CC D TM (Navarro-Llopis et al., 2008).

The similarity of efficacy levels observed when using BioLure® Med Fly and BioLure® Unipak indicated that the new presentation of the lure was as good as the previous one when used against medfly. These results confirmed those found in citrus orchards in Valencia (Navarro-Llopis et al., 2008). A recent study into the control of *Anastrepha suspensa* (Loew) performed in urban residential areas in Florida obtained very similar results (Holler et al., 2009). The main positive effects of BioLure® Unipak compared with the three-compound BioLure® Med Fly are the time-saved in field level management, because it has only one diffuser and the prolonged longevity of its action, which is 120 instead of the 45 days of BioLure® Med Fly (SUTERRA, 2007).

The results obtained in I. trial of the present work demonstrated the power of deltamethrin as a suitable substitute of DDVP, a conclusion which accords with other research in a study against medfly on mango trees in Malaga (Ros et al., 2005b). Nevertheless, in spite of the results obtained in the current study regarding alpha-cipermethrin insecticide, in other concentrations and formulations its action could be improved.

A comparative study of traps and insecticides (deltamethrin, DDVP, etc.) suggested that the effectiveness of mass trapping varies with changes in temperature (Ros et al., 2005a), findings which corroborate the results of the present work. Although deltamethrin has been tested in Girona area (chapter III of this Ph.D.) it is necessary to test it again in other areas where the use of DDVP has been also banned, because dispensers of attractants made with a slow liberation membrane have a life-span that is dependent on climatic conditions (Ros et al., 1997b) and the interaction between this fact and the effectiveness of the insecticide must be tested.

Similar results regarding the increased efficacy of systems using solid bait lures found in the current study were described in other mass trapping trials using a different methodology with Cera Trap or Maxitrap® baited with BioLure® Med Fly and the insecticide Ferag® ID TM. They were carried out in table grape vineyards (Lucas and Hermosilla, 2008b) and in citrus groves (Lucas and Hermosilla, 2008a) from Murcia. A trial performed in a citrus orchard in Mallorca demonstrated the greater efficacy of dry-baited systems when compared with the liquid ones, the best one being Tephri-trap®+BioLure® Med Fly+DDVP (Miranda et al., 2001). An important point to consider when liquid baits are used is the difficulty of distinguishing between male and female captures because the flies are dissolved in the bait solution (Lucas and Hermosilla, 2008b). This was corroborated in the present work. Another concern is that liquid protein frequently dries up quickly under warm environmental conditions (Ros et al., 1997a). For this reason, liquid baits should be replaced every few days to avoid variations in the pH content of the bait protein, which strongly reduces its attractiveness (Epsky et al., 1993). Over the 55 days of the S. trial, the liquid Cera trap® lure was refilled several times, which was time consuming. This disadvantage was confirmed in studies carried out in Tarragona, Murcia and Alicante, where Cera trap® traps were filled three times (every 17 - 18 days) in the two first locations and four times (every 26 - 28 days) in the third (Llorens et al., 2008).

5 CONCLUSIONS

Apart from the two target species *C. rosa* and *C. capitata*, other fruit fly species registered were *B. cucurbitae*, *N. cyanescens*, *D. ciliatus*, *B. zonata*, *C. catoirii* and *D. demmerezi*, in order of relevance.

The most captured species in all trials were *C. rosa* followed by *B. cucurbitae* and then *C. capitata*.

The most effective equipment for the capture of *C. rosa* and *C. capitata* were the Maxitrap® and Tephri-trap®.

The most effective baits for the attraction of *C. rosa* were BioLure® Med Fly and BioLure® Unipak. Ferag® CC D TM, BioLure® Med Fly and BioLure® Unipak obtained the same low levels of *C. capitata* captures. None of the attractants used was specific for *B. cucurbitae* and therefore, low captures were registered.

The formulation tested of the insecticide deltamethrin would be a suitable substitute for DDVP, use of which has recently been banned in the EU.

The systems including BioLure® Unipak+Tephri-trap®+DDVP and Ferag® CC D TM+Maxitrap®+DDVP performed effectively for *C. rosa* and *C. capitata*. These two systems and Cera trap® obtained the same results for *B. cucurbitae*. These results must be corroborated at a later date, using the available insecticides.

6 REFERENCES

Alonso-Muñoz, A., García-Marí, F., 2007. Comparación de cuatro tipos de mosqueros comerciales de captura de hembras y machos de Ceratitis capitata (Diptera: Tephritidae). Boletín de Sanidad Vegetal, Plagas 33**,** 399-407.

Appiah, E.F., Afreh-Nuamah, K., Obeng-Ofori, D., 2009. Abundance and distribution of the Mediterranean fruit fly Ceratitis capitata (Diptera: Tephritidae), in Late Valencia citrus orchards in Ghana. International Journal of Tropical Insect Science 29**,** 11-16.

BIOIBÉRICA, 2006. Cera Trap, un sistema eficaz y 100 % ecológico para el control de la mosca de la fruta.

Broughton, S., De Lima, C.P.F., 2002. Field evaluation of female attractants for monitoring Ceratitis capitata (Diptera : Tephritidae) under a range of climatic conditions and population levels in Western Australia. Journal of Economic Entomology 95**,** 507-512.

Cayol, J.P., Causse, R., 1993. Mediterranean fruit fly Ceratitis capitata Wiedemann (Dipt., Trypetidae) back in Southern France. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 116.

Commonwealth-Institute-of-Entomology, 1985. Distribution maps of pests, Map Nº153 Pterandrus rosa (Karsch).

Christenson, L.D., Foote, R.H., 1960. Biology of fruit flies. Annual Review of Entomology 5**,** 171-192.
De Meyer, M., 2000. Systematic revision of the subgenus Ceratitis MacLeay s.s. (Diptera, Tephritidae). Zoological Journal of the Linnean Society. 128**,** 439-467.
De Meyer, M., 2001. On the identity of the Natal fruit fly Ceratitis rosa Karsch (Diptera, Tephritidae). Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Entomologie. 71**,** 55-62.
De Meyer, M., Freidberg, A., 2005. Revision of the subgenus Ceratitis (Pterandrus) Bezzi (Diptera: Tephritidae). Israel Journal of Entomology 35-36**,** 197-315.
De Meyer, M., Robertson, M.P., Peterson, A.T., Mansell, M.W., 2008. Ecological niches and potential geographical distributions of Mediterranean fruit fly (Ceratitis capitata) and Natal fruit fly (Ceratitis rosa). Journal of Biogeography 35**,** 270-281.
Duyck, P.F., 2005. Compétition interspécifique et capacités invasives. Le cas des Tephritidae de l'île de La Réunion. Université de La Réunion.
Duyck, P.F., David, P., Quilici, S., 2004. A review of relationships between interspecific competition and invasions in fruit flies (Diptera : Tephritidae). Ecological Entomology 29**,** 511-520.
Duyck, P.F., David, P., Quilici, S., 2006. Climatic niche partitioning following successive invasions by fruit flies in La Reunion. Journal of Animal Ecology 75**,** 518-526.
Duyck, P.F., Quilici, S., 2002. Survival and development of different life stages of three Ceratitis spp. (Diptera : Tephritidae) reared at five constant temperatures. Bulletin of Entomological Research 92**,** 461-469.
EEC, 1991. Council Directive of 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC).
Epsky, N., Heath, R., Sivinski, J., Calkins, C., Baranowsi, R., Fritz, A., 1993. Evaluation of protein bait formulation for the Caribbean Fruit Fly (Diptera: Tephritidae). Florida Entomologist 76**,** 626-635.
Epsky, N.D., Hendrichs, J., Katsoyannos, B.I., Vasquez, L.A., Ros, J.P., Zumreoglu, A., Pereira, R., Bakri, A., Seewooruthun, S.I., Heath, R.R., 1999. Field evaluation of female-targeted trapping systems for Ceratitis capitata (Diptera : Tephritidae) in seven countries. Journal of Economic Entomology 92**,** 156-164.
Etienne, J., 1982. Etude systematique, faunistique et ecologique des Tephritides de La Réunion. École pratique des hautes études (Réunion).
Heath, R.R., Epsky, N.D., Dueben, B.D., Meyer, W.L., 1996. Systems to monitor and suppress Ceratitis capitata (Diptera: Tephritidae) populations. Florida Entomologist 79**,** 144-153.
Holler, T.C., Peebles, M., Young, A., Whiteman, L., Olson, S., Sivinski, J., 2009. Efficacy of the Suterra BioLure individual female fruit fly attractant packages vs. the Unipack version. Florida Entomologist 92**,** 667-669.
Katsoyannos, B.I., Heath, R.R., Papadopoulos, N.T., Epsky, N.D., Hendrichs, J., 1999. Field evaluation of Mediterranean fruit fly (Diptera : Tephritidae) female selective attractants for use in monitoring programs. Journal of Economic Entomology 92**,** 583-589.
Llorens, J.M., Matamoros, E., Lucas, A., Marín, C., Sierras, N., 2008. Integrated control of Mediterranean fruit fly Ceratitis capitata (Wied.) by mass

trapping with an enzymatic hydrolyzed protein. Control in Citrus Fruit Crops, IOBC/wprs Bulletin 38, 150-156.

Lucas, A., Fuentes, F., Hermosilla, A., 2006. Evaluación de la eficiencia de captura de adultos de mosca de la fruta (Ceratitis capitata) de diversos mosqueros y cebos, con y sin insecticida. Agrícola Vergel 294, 298-302.

Lucas, A., Hermosilla, A., 2005. Ensayo de diferentes trampas y cebos para el control de vuelo de adultos de Ceratitis capitata en cítricos. Informe de la reunión del grupo de trabajo de cítricos y subtropicales. Reuniones anuales de los grupos de trabajo fitosanitarios (MAPA, ed.), 51.

Lucas, A., Hermosilla, A., 2006. Evaluación de la eficiencia en la captura de mosca de la fruta (Ceratitis capitata) de varios mosqueros y cebos, en cultivo de cítricos. Murcia 2006 (Non published).

Lucas, A., Hermosilla, A., 2008a. Eficacia de Ceratrap y otros atrayentes y mosqueros, en el control de mosca de la fruta (Ceratitis capitata) en cítricos. Agrícola Vergel 390, 159-166.

Lucas, A., Hermosilla, A., 2008b. Eficacia de Ceratrap y otros atrayentes y mosqueros, en el control de mosca de la fruta (Ceratitis capitata) en uva de mesa. Agrícola Vergel 319, 304-310.

Lucas, A., Hermosilla, A., 2008c. Eficacia de diferentes atrayentes y mosqueros para el control de mosca de la fruta (Ceratitis capitata) en uva de mesa. Agricola Vergel 27, 260-268.

Lucas, A., Hermosilla, A., 2008d. Evaluación de la eficiencia en la captura de mosca de la fruta (Ceratitis capitata) de varios mosqueros y cebos, en cultivo de cítricos. Levante Agrícola 390, 169-178.

Miranda, M.A., Alonso, R., Alemany, A., 2001. Field evaluation of Medfly (Dipt., Tephritidae) female attractants in a Mediterranean agrosystem (Balearic Islands, Spain). Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 125, 333-339.

Navarro-Llopis, V., Alfaro, F., Dominguez, J., Sanchis, J., Primo, J., 2008. Evaluation of traps and lures for mass trapping of Mediterranean fruit fly in citrus groves. Journal of Economic Entomology 101, 126-131.

Piñol, R., 2009. Ceratrap®: sistema eficaz ecológico contra Ceratitis. Phytoma España 213, 31.

Quilici, S., Duyck, P.F., Rousse, P., Gourdon, F., Simiand, C., Franck, A., 2005. La mouche de la pêche sur mangue, goyave, etc. Phytoma, la défense des végétaux. 584, 44-47.

Quilici, S., Franck, A., 1999. The Natal fruit fly. Technical Bulletins on the crop pests of the Indian Ocean Region. CIRAD.

Quilici, S., Jeuffrault, E., 2001. Plantes-Hôtes des mouches des fruits, Maurice, Réunion, Seychelles.

Ros, J.P., Castillo, E., Crespo, J., Latorre, Y., Martin, P., Miranda, M.A., Moner, P., Sastre, C., 1997a. Evaluación en campo de varios atrayentes sintéticos para la captura de hembras de la mosca mediterránea de la fruta Ceratitis capitata Wied. (Díptera: Tephritidae). Boletín de Sanidad Vegetal, Plagas 23, 393-402.

Ros, J.P., Castillo, E., Wong, E., Olivero, J., Rubio, J.R., Marquez, A.L., 2005a. Development of traps and killing agents to improve the mass trapping techinique against Ceratitis capitata Wied. and Bactrocera oleae Gmel. (Diptera: Teprhitidae). Development of improved attractants and their integration into Fruit Fly SIT management programmes, Vienna 5-7 May 2005, pp. 27.

Ros, J.P., Wong, E., Castro, V., Castillo, E., 1997b. La Trimetilamina: un efectivo potenciador de los atrayentes putrescina y acetato amónico para capturar las hembras de la mosca mediterránea de la fruta Ceratitis capitata Wied. (Diptera: Teprhitidae). Boletín de Sanidad Vegetal, Plagas 23**,** 515-521.

Ros, J.P., Wong, E., Olivero, J., Rubio, J.R., Marquez, A.L., Castillo, E., Blas, P., 2005b. Desarrollo de atrayentes y mosqueros para su integración en los programas de trampeo masivo contra la mosca de la fruta (Ceratitis capitata Wied.) y la del olivo (Bactrocera oleae Gmel.). Boletín de Sanidad Vegetal, Plagas 31**,** 599-609.

Ryckewaert, P., Deguine, J.P., Brevault, T., Vayssieres, J.F., 2010. Fruit flies (Diptera: Tephritidae) on vegetable crops in Reunion Island (Indian Ocean): state of knowledge, control methods and prospects for management. Fruits 65**,** 113-130.

Steiner, L.F., 1957. Low-cost plastic fruit fly trap. Journal of Economic Entomology 50**,** 508-509.

SUTERRA, 2007. Productos para el control de Ceratitis capitata.

Vayssières, J.F., Coubès, M., 1999. The Melon Fly. Technical Bulletins on the crop pests of the Indian Ocean Region. CIRAD.

Vinaches, P., Marco, F., Llorens, J.M., 2005. Ensayo para determinar la eficacia de captura para Ceratitis capitata Wied. de diversas trampas comerciales con los mismos atrayentes. Informe de la reunión del grupo de trabajo de cítricos y subtropicales. Reuniones anuales de los grupos de trabajo fitosanitarios.**,** 52.

Weems, H.V., Fasulo, T.R., 2007. Natal fruit fly. http://creatures.ifas.ufl.edu/fruit/tropical/natal_fruit_fly.htm.

White, I.M., Elson-Harris, M.M., 1992. Fruit flies of economic significance: Their identification and bionomics. Wallingford, Oxon, CAB International.

5. CHAPTER III: Insecticides for use in mass trapping control technique for *Ceratitis capitata*

INDEX

1 INTRODUCTION 131

2 MATERIAL AND METHODS 131

2.1 COMPARATIVE TRIALS: SELECTION OF THE INSECTICIDE AND LOCATION OF THE INSECTICIDE IN THE TRAP 131

2.2 MASS TRAPPING TRIAL 134

3 RESULTS 136

3.1 FORMULATION TRIAL (I): DDVP AND DELTAMETHRIN AT DIFFERENT DOSAGES AND SOLVENT MEDIUMS 136

3.2 FORMULATION TRIAL (II): DDVP, DELTAMETHRIN AND CHLORPYRIFOS AT DIFFERNENT DOSAGES 140

3.3 POSITION TRIAL: LOCATION OF THE INSECTICIDE IN THE TRAP 144

3.4 MASS TRAPPING TRIAL 147

4 DISCUSSION 150

5 CONCLUSIONS 153

6 REFERENCES 154

1 INTRODUCTION

Mass trapping is an alternative methodology to the exclusive use of chemical products in the control of *Ceratitis capitata* (Wiedemann), being completely compatible with integrated pest management and also helping to reduce chemical residues in fruits.

As mentioned in the general introduction of this Ph.D. any insecticide used in mass trapping must comply with current European legislation and therefore, must be listed in Annexe I of the Directive 91/414/CEE (EEC, 1991). The insecticide used over the last few years in mass trapping has been dichlorvos (2.2-dichlorovinyl dimethyl phosphate, DDVP) but this has recently been removed from the European normative. In 2010 its use was authorized under exceptional circumstances because this method without a retention system would allow the flies to escape from the traps and therefore, the effectiveness of the method would decrease.

The need to find and optimize a new insecticide for mass trapping has recently promoted a number of trials in the Girona area (non published data).

The aims of the present study were:

1. To find an insecticide and formulation for used in the mass trapping of medfly, which is at least as effective as DDVP.
2. To identify the optimal location of the insecticide in the trap.
3. To ascertain the efficacy of the prototype carrying the selected insecticide for mass trapping of medfly.

2 MATERIAL AND METHODS

2.1 COMPARATIVE TRIALS: SELECTION OF THE INSECTICIDE AND LOCATION OF THE INSECTICIDE IN THE TRAP

In order to select the insecticide and its optimal location in the trap, three trials were performed in commercial plots located in the Baix Empordà fruit growing area (Girona) (Table 11).

The first trial (identified as F. (I)) compared varying dosages and formulations of deltamethrin. The second (identified as F. (II)) compared different insecticides and dosages. Both trials were carried out in 2007; each in two different plots due to the reduction in the medfly population in the plots where they were first located (Table 11). The third (identified as P.) compared a range of positions of the insecticide in the trap, and was carried out in one plot in 2009 (Table 11).

Seven treatments were evaluated in F. (I), six in F. (II) and two in P. trial (Table 12). All treatments were impregnated by movement of the base of the lid to disperse a known amount of the product dissolved in aqueous medium, methylene chloride (with easily evaporation) or xylene (with low volatility) (Table 12), and agitating the lid until its entire inner surface was completely impregnated. However, DDVP and DM 20 B were used in membrane diffusers and DM 2 was used in powder form in the lid. All these treatments were formulated by SEDQ S.L. (Barcelona, Spain), with the exception of DM 20 in trial F. (II), which was a commercial formulation from another company in which the solvent medium was unspecified.

Table 11. Characteristics of the plots where the trials to select the insecticide (F. (I) and F. (II)) and the location of the insecticide in the trap (P.) were carried out.

TRIAL	SURFACE (ha)	CROP	VARIETY	PLANTATION FRAME (m)	HARVEST PERIOD
F. (I)	0.70	Peach	‘Rich Lady’ & ‘S. Rich’	5 x 2	Mid July & late July
	2.73	Apple	‘Smoothee’	3.8 x 1.2	Mid September
F. (II)	2.88	Peach	‘Summer Rich’	5 x 2.5	Late July
	2.73	Apple	‘Smoothee’	3.8 x 1.2	Mid September
P.	0.62	Peach	‘Summer Rich’	4.5 x 2	Late July

Control treatments used in the first two trials were DDVP and deltamethrin 300 mg manufactured in 2007 (DM 300-07), because they had produced the best results in previous studies performed in the same area in 2006. Preserved samples of the active ingredient and formulation from the 2006 stock were used in the trial F. (I) (DM 300-06), in order to verify its effectiveness after one year of storage in hermetically sealed containers at a temperature of - 20ºC. Other treatments tested in this trial focused on varying dosages of the insecticide and solvents: in an aqueous medium (DM 50 A), in xylene (DM 25 X), in methylene chloride at lower concentration of the active ingredient (DM 20) and this concentration but with the insecticide in a different location (DM 20 B).

In trial F. (II), the formulations compared with the controls were: a treatment based on the active ingredient chlorpyrifos 100 mg dissolved in an aqueous medium (CP 100), deltamethrin 20 mg with an unknown medium (DM 20), a low concentration of the same active ingredient cited above in powder form (DM 2) and the same formulation in xylene medium (DM 2 X).

Trial P. examined the treatment with deltamethrin 12 mg in an aqueous medium created by movement impregnation on the base of the lid and the same formulation inside the trap.

In all three trials, the trap model used was Maxitrap® (Probodelt, S.L., Amposta, Spain) baited with FERAG® CC DDD TM (SEDQ S.L.) comprised of ammonium acetate, trimethylamine and diaminoalkane in different dispensers.

Table 12. Identification of the treatments used in the comparative studies.

TRIAL	TREATMENT	DOSAGE OF ACTIVE INGREDIENT (mg)	PRESENTATION MODE	POSITION
F. (I)	DDVP	FERAG ID TM 320	dispenser	base of trap
	DM 300-07	Deltamethrin 300	methylene chloride medium	lid
	DM 300-06	Deltamethrin 300	methylene chloride medium	lid
	DM 50 A	Deltamethrin 50	aqueous medium	lid
	DM 25 X	Deltamethrin 25	xylene medium	lid
	DM 20	Deltamethrin 20	methylene chloride medium	lid
	DM 20 B	Deltamethrin 20	dispenser	base of trap
F. (II)	DDVP	FERAG ID TM 320	dispenser	base of trap
	DM 300-07	Deltamethrin 300	methylene chloride medium	lid
	CP 100	Chlorpyrifos 100	aqueous medium	lid
	DM 2	Deltamethrin 2	powder	lid
	DM 20	Deltamethrin 20	medium not specified	lid
	DM 2 X	Deltamethrin 2	xylene medium	lid
P.	DM 12 L	Deltamethrin 12	aqueous medium	lid
	DM 12 B	Deltamethrin 12	aqueous medium	base of trap

The experimental design of the first two trials consisted of fully randomized blocks with four replicates. The last trial comprised five blocks, each with four repetitions per treatment (i.e. a total of 20 replicates per treatment). Two clockwise rotations were made each week in order to diminish the effect of the trap position in each block. One cycle covered the period during which a treatment needed to be located in its original position. Four complete cycles were carried out in trials F. (I) and F. (II), lasting 98 and 85 days, respectively. The first two cycles were conducted in the peach orchard and the last two in the apple orchards (Table 11). Two complete cycles of 27 and 28 days and one incomplete cycle of 23 days were carried out in trial P., which lasted a total of 78 days.

Traps were hung on 13th August, 2007, 14th August 2007 and 29th August, 2009 in F. (I), F. (II) and P. trials, respectively at a height of 1.5 m in the tree canopy, with a South Easterly orientation and with a trap separation of 20 m in the first two trials and 8 m in the last.

In trials F. (I) and F. (II), the number of live and dead individuals per trap was recorded at each trap rotation date (twice per week), while in the third trial, they were recorded only at the end of each cycle. For all trials, the total number of live and dead flies per trap for each treatment and cycle was transformed to a relative value i.e. the percentage of the total captures in one cycle registered for each treatment. The relative number of captures was obtained using the arcsin transformation (arcsine of the square root of the percentage), and subjected to the Levene test of homogeneity of variance. An ANOVA for fully randomised blocks, followed by a Tukey's studentized test for means separation was carried out, using the Enterprise Guide of the SAS program.

Charts were then drawn up to show total captures (dead and live), the percentage of dead and live individuals and the accumulated value of dead and live adults for each cycle. The effectiveness of the active ingredients and formulations used was evaluated in relation to the lower percentage of live flies and the higher percentage of dead individuals captured.

Meteorological data recorded during the study periods was provided by the Catalonian Meteorological Service Station (DAR, 2010), through two weather stations located in the survey area, in La Tallada d'Empordà and Torroella de Montgrí (Girona).

2.2 MASS TRAPPING TRIAL

This trial was carried out in a commercial apple plot located in Baix Empordà fruit growing area (Girona). Plot dimensions were 2.37 ha, of which 1.48 ha contained the 'Fuji' variety and 0.89 ha 'Granny Smith'. The second variety was not intercropped but clustered in the Southern part of the plot. The distance between trees was 3.80 m x 1.20 m and harvesting took place from 9th October to 2nd November, 2009.

The equipment used was Maxitrap® traps baited with FERAG® CC D TM attractants, with only one dispenser containing the three compounds: ammonium acetate, trimethylamine and diaminoalkane. The active ingredient and the insecticide formulation were chosen after examining the results of the trials performed over the three previous years: a formulation of deltamethrin 12 mg. However, a new prototype designed by SEDQ S.L. to carry the formulation was also tested for commercially viability (Figure 35). This prototype consisted of a plastic device with the insecticide installed by movement impregnation to ensure that it covered the entire inner part of the lid. This can be produced on a large scale as a high quality product.

Figure 35. Traps provided with the food lure for medfly and lids provided with the prototype which carries the insecticide tested. Photo: E. Peñarrubia.

One hundred traps were hung on 28th August, 2009 in a homogenous distribution across the plot (Figure 36).

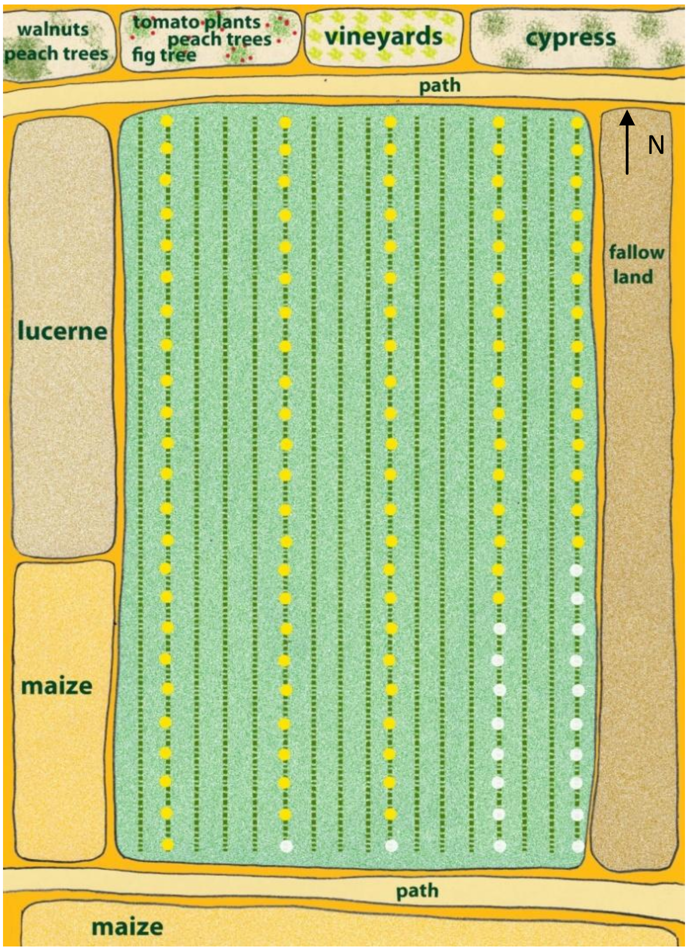


Figure 36. Diagram of the plot and trap positions following a homogeneous distribution. Each yellow dot corresponds to a trap used in the trial. The SE extreme had a background of almost null captures and trees were recently planted; however, the grower hung 20 traps (white dots) baited with DDVP, which produced very few captures. These traps did not belong to the trial. Drawing: Maria Beitia.

Traps were placed 14 m apart and numbered to identify their position and distribution, thus enhancing the construction of spatial graphs. Each trap was checked weekly and the numbers of dead and live captures and male and female captures were registered.

The grower and the technician responsible for the plot decided to spray a chemical treatment when the population captured was high enough to justify it, i.e. five females/trap/day, the threshold established in the trial area (Batllori et al., 2008). Therefore, when an increase in captures was detected on 29th September, a chemical treatment with a formulation based on Lambda-cyhalothrin 10% was performed, although no damage had been found in the two previous evaluations of fruits.

After the harvest had finished, traps were kept hung on trees, until captures fell to zero, in order to diminish the population present in the plot and to avoid their dispersion to nearby orchards. The trial had a monitoring period lasting 133 days.

The efficacy analysis of the mass trapping using the prototype of insecticide was evaluated in two different ways through:

a) Damage analysis consisting of four fruit evaluations: the first, on the day before the placing of the traps, the second a week later, the third three weeks after that and the final one a fortnight later, coinciding with the harvest. One thousand fruits/ha (10 fruits/tree on 100 randomly chosen trees) were checked in each evaluation. Fruits which were suspected of containing medfly larvae were picked and checked under stereomicroscope (binocular magnifying glass) in the laboratory.

b) Population dynamics were monitored by trapping adults. Captures were expressed as the number of adults/trap/day (F/T/D) and the data was then fed into the population dynamic graph. The Enterprise Guide of the SAS program was used to construct a spatial graph using data for the first six weeks of the trial up to the harvest to show the distribution of captures at plot level.

Meteorological data was provided by the Catalonian Meteorological Service Station (DAR, 2010), through the weather station located 830 m from the survey plot in La Tallada d’Empordà (Girona).

3 RESULTS

3.1 FORMULATION TRIAL (I): DDVP AND DELTAMETHRIN AT DIFFERENT DOSAGES AND SOLVENT MEDIUMS

The total number of live and dead adults caught in all the traps in the first orchard was 937 and 4,266, respectively and in the second orchard, 289 and 3,462 flies.

Live captures varied significantly according to the treatments in each cycle (Table 13). DDVP achieved significantly the lowest mean percentage of live flies in the first cycle, in which the highest number of captures was registered (826 live flies). References DM 300-07, DM 300-06 and DM 20 obtained the lower quantity of live flies just below DDVP, with no significant differences between them, except for the second cycle. No significant differences were found between treatments DM 300-07 and DM 300-06 and the number of live flies recorded over the 98 days evaluated. DM 50 A was not significantly different from the treatment with half dose and the other solvent medium, DM 25 X in any of the cycles.

In three out of four cases, the formulation located on the base of the traps as a dispenser, DM 20 B, captured a higher percentage of live flies, although it was statistically different from all the other treatments only in the last cycle (Table 13). This treatment was compared with another with the same dosage but a different presentation mode, DM 20, formulated with methylene chloride and significant differences were identified in the second half of the period studied.

Table 13. Mean relative number of live captures per cycle in each treatment of trial F. (I). Cycles 1 and 2 were carried out in the peach orchard, and cycles 3 and 4 in the apple orchard. Values followed by the same letter in the same column are not significantly different (Tukey's studentized test, P < 0.05) n = 4.

TREATMENT	CYCLE							
	1		2		3		4	
DDVP	0.72	*c*	0.00	*b*	1.94	*d*	3.59	*bc*
DM 300-07	9.69	*b*	10.87	*ab*	7.32	*bcd*	0.00	*c*
DM 300-06	14.28	*ab*	7.10	*b*	5.65	*bcd*	4.17	*bc*
DM 50 A	17.15	*ab*	29.78	*a*	18.88	*abc*	6.07	*bc*
DM 25 X	16.36	*ab*	24.84	*a*	24.34	*ab*	18.07	*bc*
DM 20	17.91	*ab*	10.53	*a*	5.81	*c*	3.59	*bc*
DM 20 B	27.46	*a*	18.66	*a*	37.48	*a*	65.56	*a*
P-value	*p<0.0001*		*p=0.0002*		*p<0.0001*		*p<0.0001*	
Total number of live catches	826		111		241		48	

Over the 98 days evaluated, no significant differences were found in captures of dead medflies between the controls (DDVP and DM 300-07) and the other treatments, with the exception of DM 20 B, which registered the lowest percentage of dead flies (Table 14). Comparing treatments using equal dosages but different presentation modes (DM 20 and DM 20 B), throughout the study as whole, significant differences were found, except in the last cycle (Table 14).

Table 14. Mean relative number of dead captures per cycle in each treatment of trial F. (I). Cycles 1 and 2 were carried out in the peach orchard, and cycles 3 and 4 in the apple orchard. Values followed by the same letter in the same column are not significantly different (Tukey's studentized test, P < 0.05) n = 4.

TREATMENT	CYCLE							
	1		2		3		4	
DDVP	18.77	*ab*	13.99	*a*	19.17	*a*	18.29	*a*
DM 300-07	17.36	*ab*	26.38	*a*	16.43	*a*	14.89	*ab*
DM 300-06	16.83	*ab*	18.48	*a*	16.84	*a*	19.54	*a*
DM 50 A	8.26	*bc*	13.30	*a*	12.60	*a*	18.46	*a*
DM 25 X	20.08	*ab*	12.90	*ab*	14.73	*a*	13.08	*ab*
DM 20	20.88	*a*	17.96	*a*	20.45	*a*	14.27	*ab*
DM 20 B	2.03	*c*	2.62	*b*	4.00	*b*	6.36	*b*
P-value	*p<0.0001*		*p=0.0005*		*p<0.0001*		*p=0.0122*	
Total number of dead catches	3,743		523		2,720		742	

The mean relative number of live and dead captures was analysed statistically and grouped by cycles (Tables 13 and 14). The differences observed over the study period for a specific treatment is also shown in a chart (Figure 37).

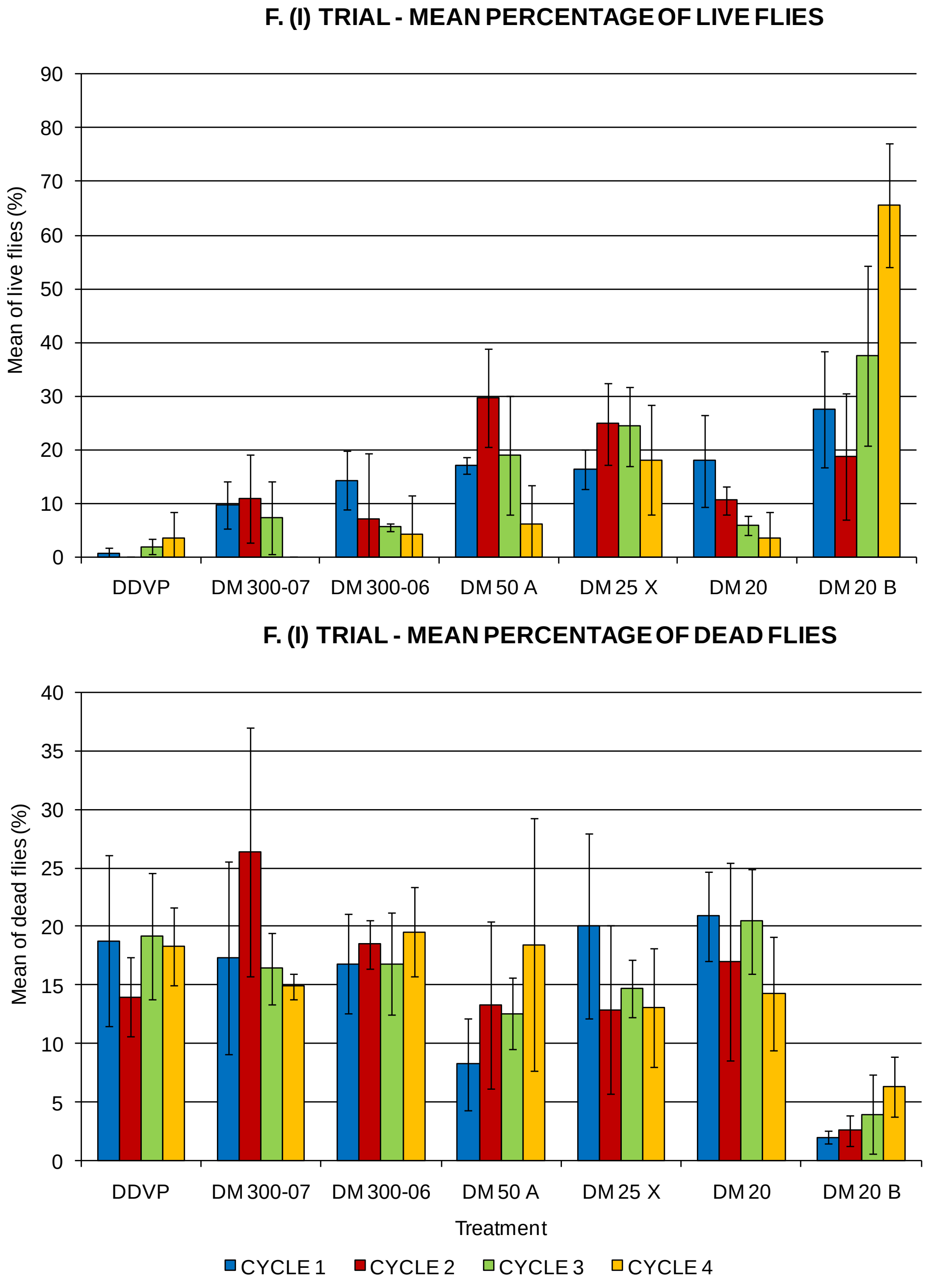


Figure 37. Mean percentage of live and dead flies found inside the traps in each cycle.

Total captures of live and dead flies varied throughout the entire trial period (Figure 38) and in the second cycle these figures were more than seven times lower than in the first one, as a result of which the traps were moved to another orchard where the population captured level in monitoring traps was higher. This change was effected in order to increase the number of captures in the remaining cycles and thus, increase the reliability of the data. After moving this trial to other orchard, dead captures were more than 5 times higher than in the previous period.

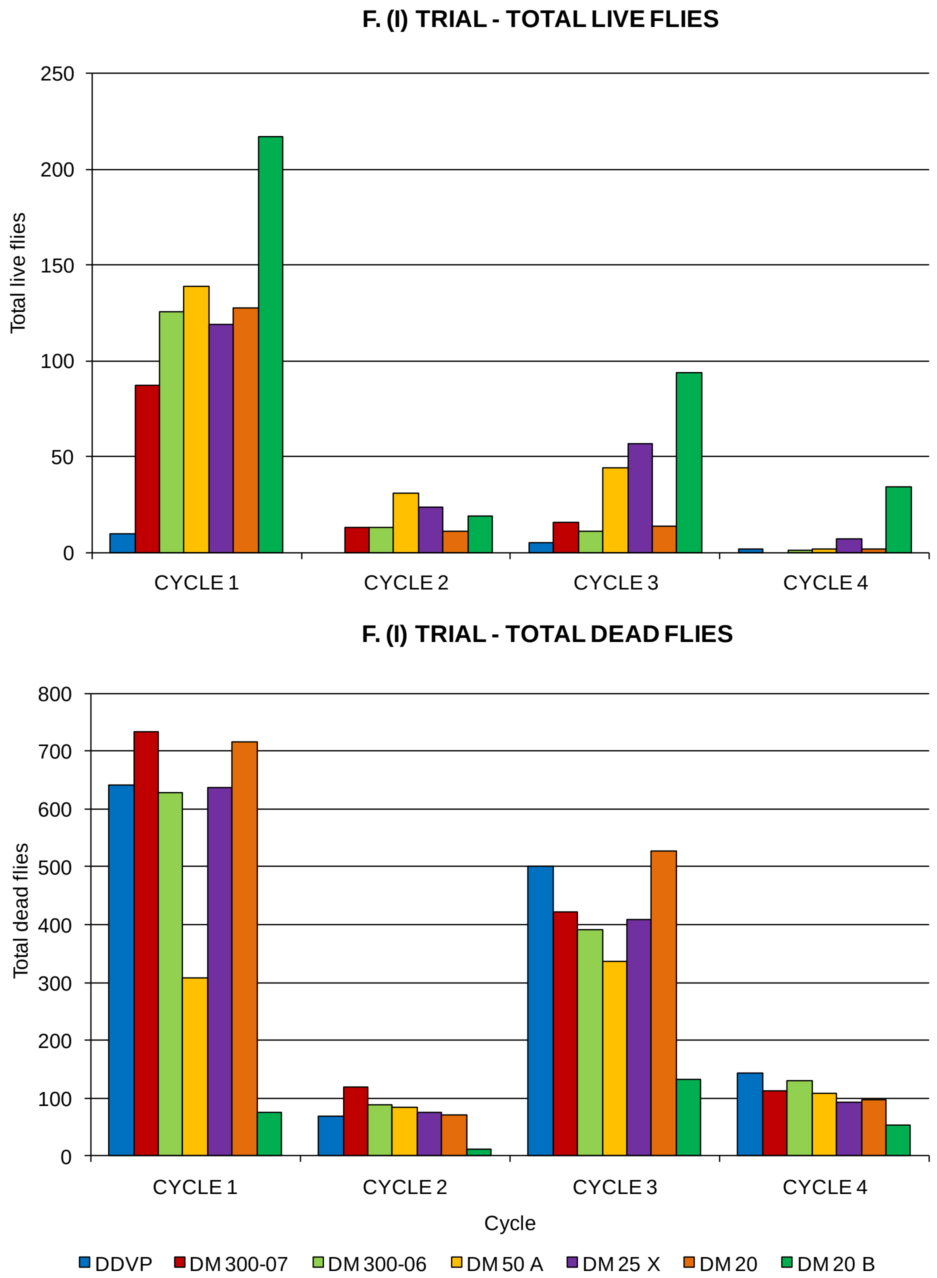


Figure 38. Total number of flies found live and dead inside the traps in each cycle.

Charts of accumulated captures confirmed visually that DM 20 B left the highest quantity of live flies and the lowest level of dead ones over the entire trial period. This treatment was followed by DM 50 A. Taking into account the accumulated number of dead flies captured, DM 300-06, DM 25 X and DM 20 appear to produce similar results but DM 25 X left more live individuals (Figure 39).

Climatic data registered in trial F. (I) could be summarized as follows: maximum daily temperature ranging from 31.6ºC to 10.3ºC, daily mean temperature between 25ºC and 1.4ºC, and daily minimum temperature between 20.4ºC and - 6.6ºC. Accumulated rainfall over the entire trial period was 117.8 mm, with a daily maximum of 39.6 mm on 10th October, 2007.

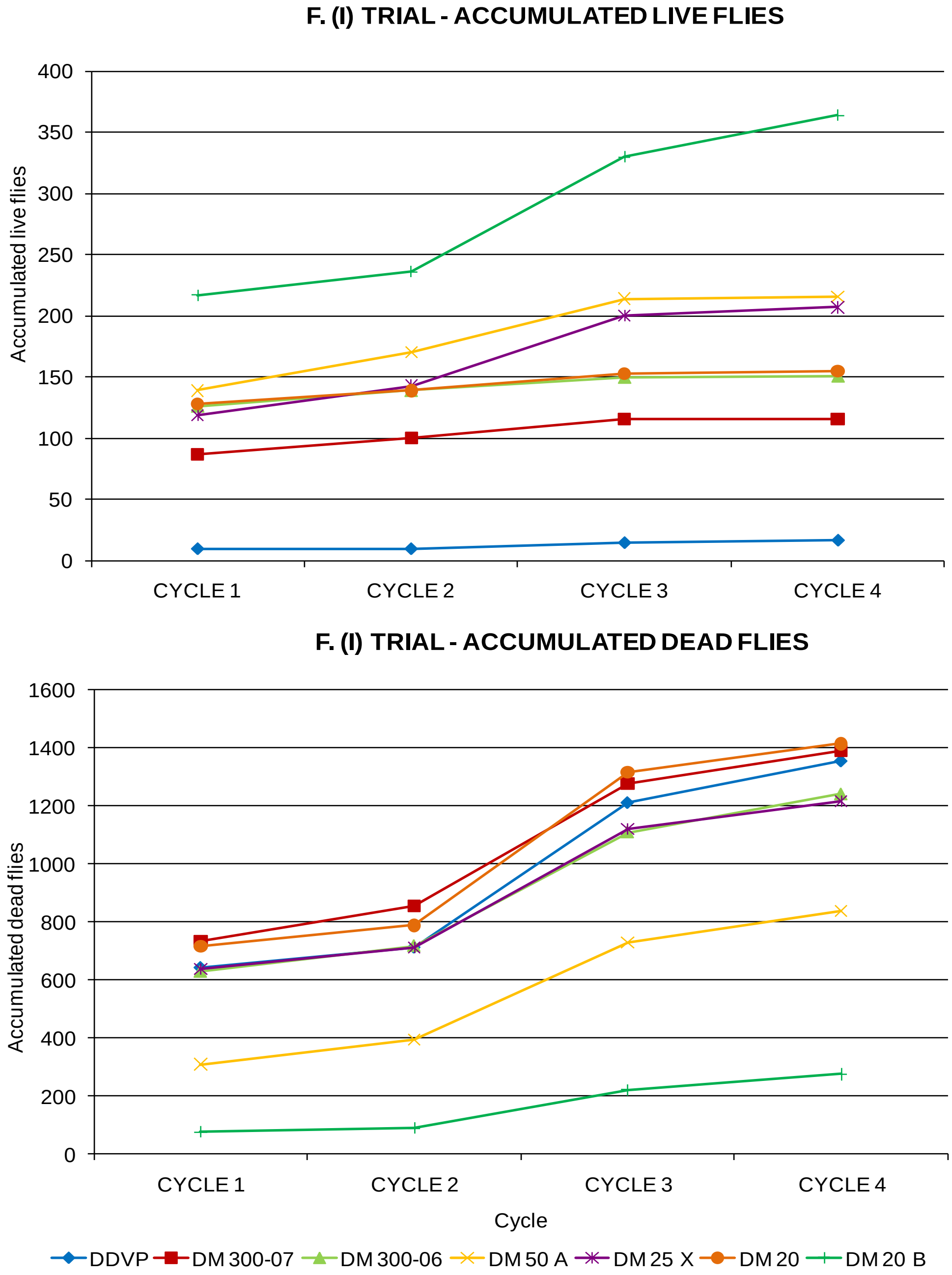


Figure 39. Accumulated number of live and dead flies captured with each treatment over cycles.

3.2 FORMULATION TRIAL (II): DDVP, DELTAMETHRIN AND CHLORPYRIFOS AT DIFFERNENT DOSAGES

The total number of live and dead adults caught in all the traps in the first orchard was 203 and 2,501, respectively and in the second 285 and 9,252 flies.

Statistical analysis confirmed that there were no differences between DDVP, DM 2 and DM 20 throughout the entire period of the study, except in the last cycle. These insecticides provided a lower percentage of live individuals although differences between them and the other treatments were not always significant (Table 15). Formulations DM 300-07, CP 100 (formulated in basis to chlorpyrifos) and DM 2 X did not differ statistically in any cycle and allowed a higher percentage of flies to remain live inside the traps. The same dosage (2 mg of deltamethrin) was compared using two presentations, DM 2 formulated in powder and DM 2 X formulated using a xylene medium. They were significantly different only in the first half of the study period (Table 15).

Table 15. Mean relative number of live captures per cycle in each treatment of trial F. (II). Cycles 1 and 2 were carried out in the peach orchard and cycles 3 and 4 in the apple orchard. Values followed by the same letter in the same column are not significantly different (Tukey's studentized test, P < 0.05) n = 4.

TREATMENT	CYCLE							
	1		2		3		4	
DDVP	2.22	*c*	0.00	*b*	9.54	*b*	6.61	*b*
DM 300-07	18.93	*ab*	17.36	*ab*	14.75	*ab*	13.42	*ab*
CP 100	21.74	*ab*	20.83	*ab*	17.01	*ab*	38.05	*ab*
DM 2	8.74	*bc*	3.13	*b*	14.98	*ab*	8.95	*a*
DM 20	9.82	*bc*	2.78	*b*	12.13	*ab*	9.67	*ab*
DM 2 X	38.54	*a*	55.90	*a*	32.59	*a*	23.30	*ab*
P-value	*p=0.0004*		*p=0.0026*		*p=0.0582*		*p=0.0270*	
Total number of live catches	179		24		218		67	

When compared with all the other treatments, in the first three cycles, control DDVP showed no significant differences with regard to dead captures but in the last cycle, a difference was found with the other control DM 300-07 and with the treatments CP 100 and DM 2 X (Table 16). The control DM 300-07 produced the highest mean percentage of dead flies but in no cycle there was any significant difference from DM 2. Treatments differing in the form of presentation (DM 2 and DM 2 X) did not differ statistically in any cycle analysed in respect of the number of dead flies captured (Table 16).

Table 16. Mean relative number of dead captures per cycle in each treatment of trial F. (II). Cycles 1 and 2 were carried out in the peach orchard and cycles 3 and 4 in the apple orchard. Values followed by the same letter in the same column are not significantly different (Tukey's studentized test, P < 0.05) n = 4.

TREATMENT	CYCLE							
	1		2		3		4	
DDVP	19.18	*ab*	22.39	*ab*	18.07	*ab*	14.53	*bc*
DM 300-07	26.16	*a*	27.12	*a*	20.27	*a*	20.67	*a*
CP 100	15.73	*ab*	8.62	*b*	11.03	*b*	9.56	*d*
DM 2	12.38	*ab*	22.96	*ab*	19.79	*a*	20.39	*ab*
DM 20	11.87	*b*	10.46	*b*	14.08	*ab*	13.94	*cd*
DM 2 X	14.68	*ab*	9.45	*b*	16.76	*ab*	20.92	*a*
P-value	*p=0.0143*		*p=0.0037*		*p=0.0090*		*p<0.0001*	
Total number of dead catches	2,066		435		6,452		2,800	

As in the previous trial, differences over the full period observed among the mean relative numbers of live and dead catches of each treatment is shown in a chart (Figure 40).

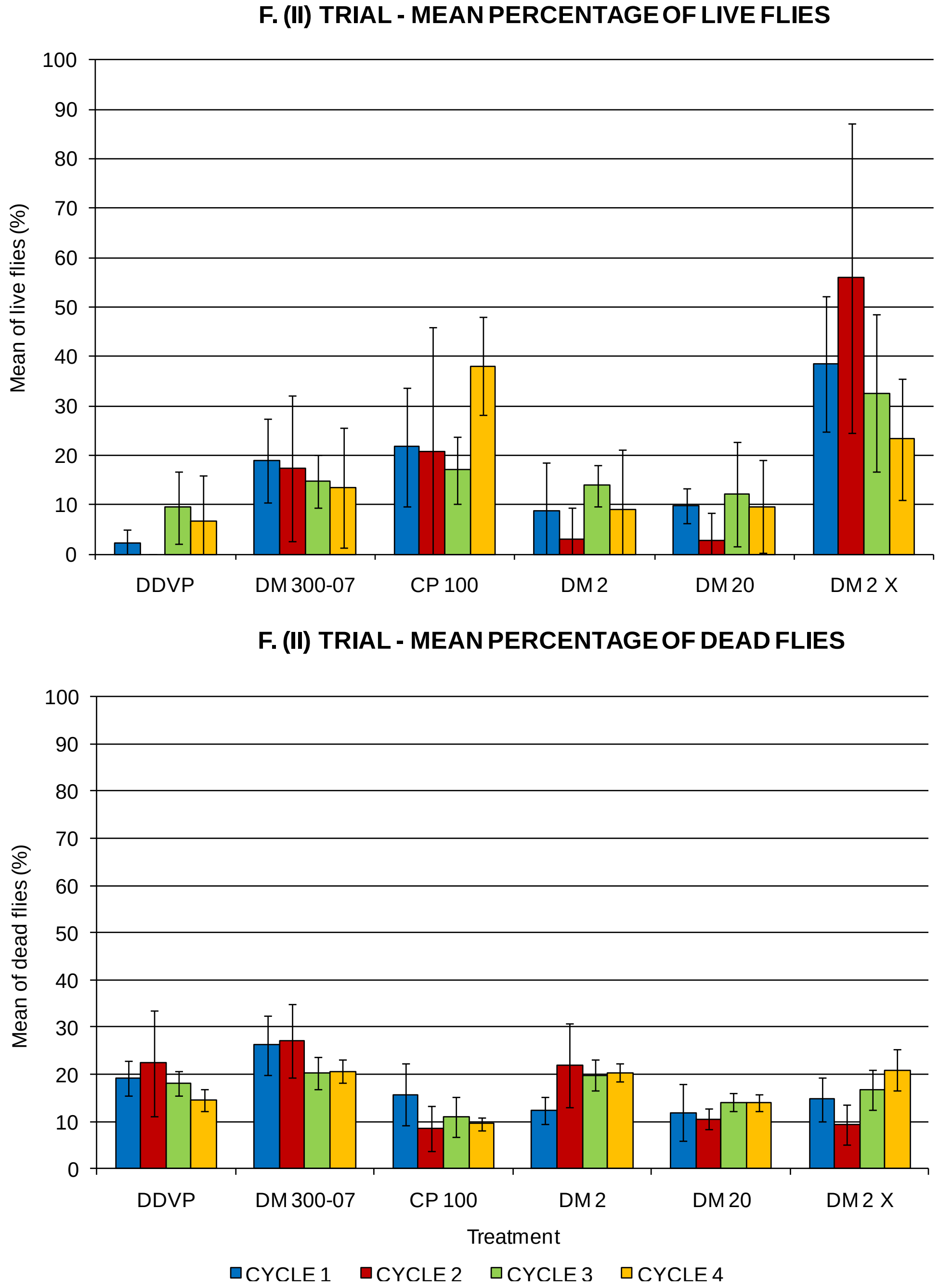


Figure 40. Mean percentage of flies found live and dead inside the traps in each cycle.

Captures of live and dead medflies varied over the full period (Figure 41). In the second cycle, captures were almost five times lower than in the previous one and, as in trial F. (I), this finding triggered the movement of the traps to another orchard where the population capture levels in monitoring traps was higher.

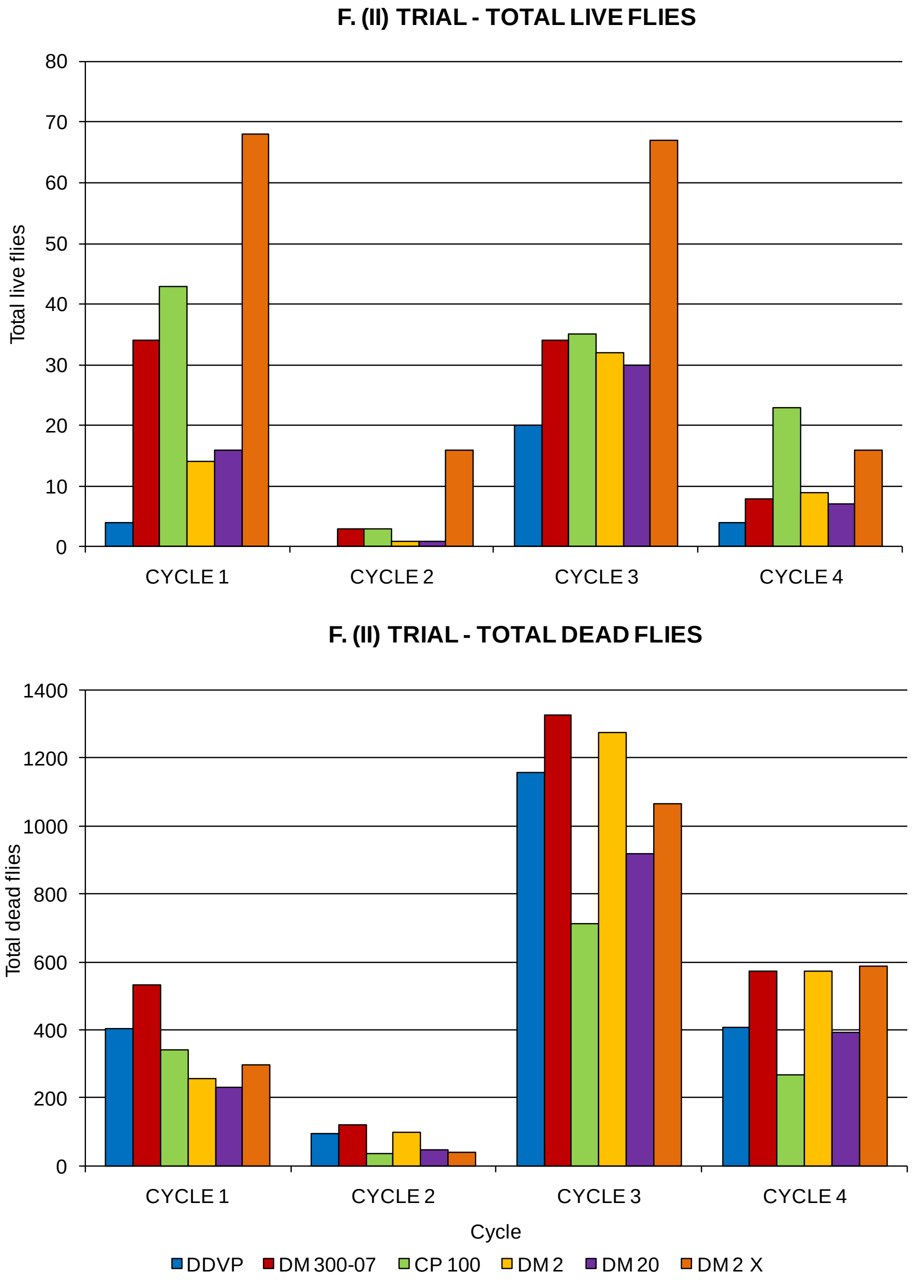
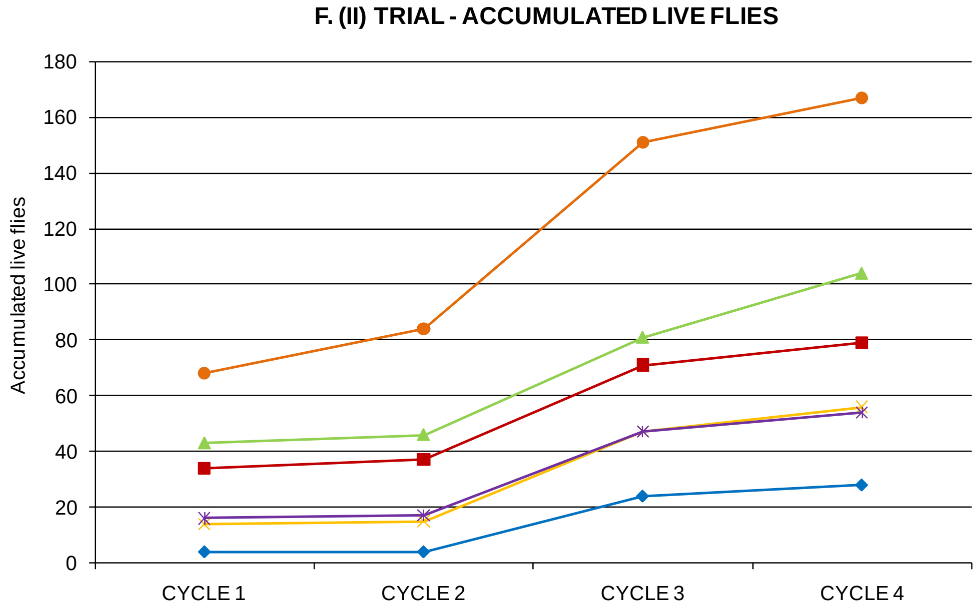


Figure 41. Total number of flies found live and dead inside the traps in each cycle.

The chart of accumulated live individuals showed that DM 2 X left more flies than any other formulations (Figure 42) and the best results were obtained with the control DDVP, followed by DM 2 and DM 20. Accumulated captures of dead flies at the end of the trial confirmed the best performers to be DM 300-07 followed by DM 2 (Figure 42), as has been described in the statistical analysis above.

Climatic data registered in trial F. (II) showed daily maximum temperature varying between 31.6 and 13.6ºC, daily mean temperature between 25 and 9.8ºC, and daily minimum temperature between 20.4 and 2.3ºC. Accumulated rainfall over the entire period was 116.2 mm and the maximum daily precipitation was the same as in the previous trial.



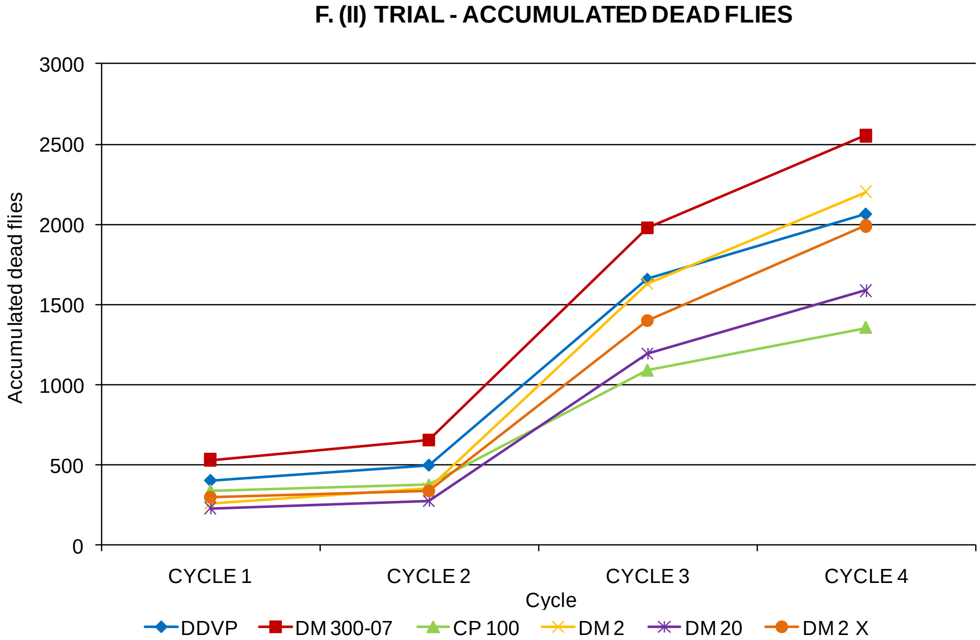


Figure 42. Accumulated number of live and dead flies captured with each treatment over cycles.

3.3 POSITION TRIAL: LOCATION OF THE INSECTICIDE IN THE TRAP

In the first cycle significant differences were observed between both positions of insecticides in the inner part of the trap for its use in mass trapping technique (Table 17). When each cycle was finished, traps were checked and all adults found were dead. Data from all traps showed that the number of adults registered dead decreased from 1,273 to 17 between the first and last cycle (Figure 43).

Table 17. Mean relative number of dead captures per cycle in each treatment of trial P. and their standard deviation. Values from each cycle followed by the same letter are not significantly different (Tukey's studentized test, $P < 0.05$) n = 20.

CYCLE	TREATMENT	MEAN RELATIVE NUMBER OF DEAD FLIES	STANDARD DEVIATION
1	DM 12 L	0.76 b	0.03
	DM 12 B	0.81 a	0.03
2	DM 12 L	0.78 a	0.08
	DM 12 B	0.79 a	0.08
3	DM 12 L	0.96 a	0.36
	DM 12 B	0.62 a	0.36

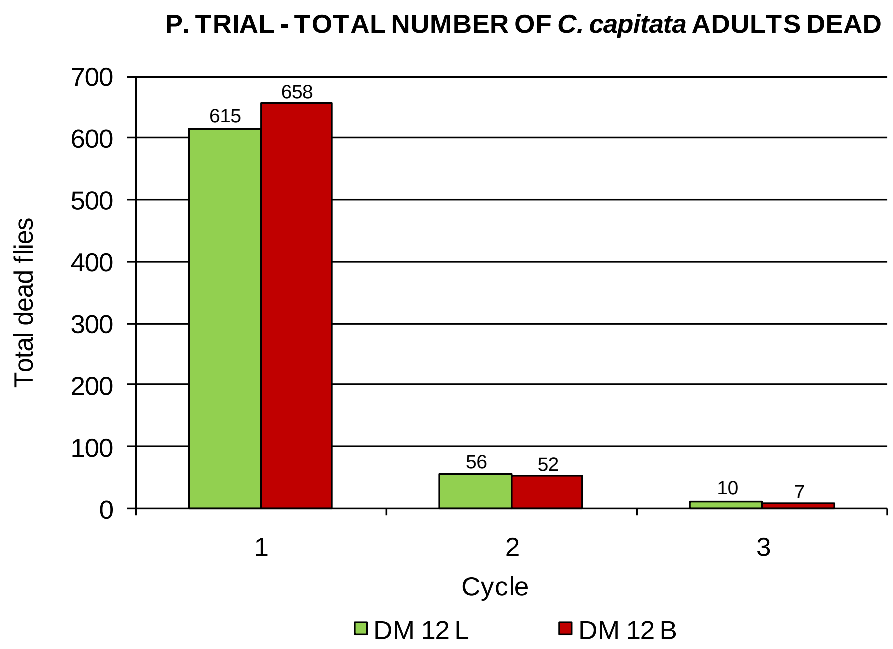


Figure 43. Total captures level of dead adults in all blocks and repetitions.

The results of the first two cycles, with higher number of captures and lower standard deviation, showed equivalent percentages of dead adults of medfly for each treatment (Figure 44), although in the first cycle there were significant differences between the two treatments.

Extremely low captures were registered in the last cycle (Figure 43) and a high standard deviation was calculated in the percentage of medfly found in this period with each treatment (Figure 44). Due to these two factors, despite the low percentage of individuals found in the third cycle, DM 12 L registered twice as many as DM 12 B. This data has not been factored into the calculations.

Statistical analysis identified small differences only in the first cycle reflecting the differing positions of the insecticide in the trap (Table 18).

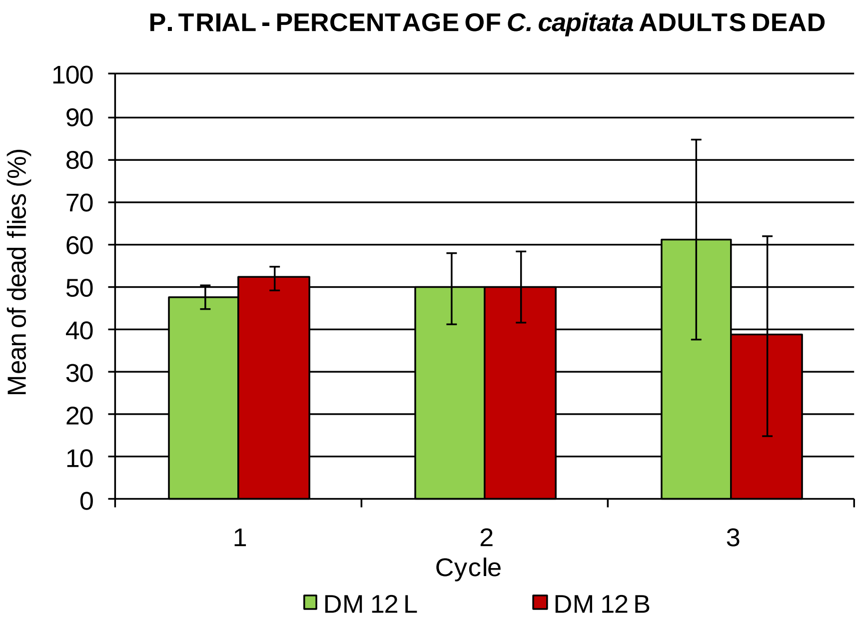


Figure 44. Percentage of adults found dead and their standard deviation. n = 20

Table 18. Anova results taking into account the comparison between the two treatments tested in trial P., for each cycle ($P < 0.05$) n = 20.

CYCLE	F-Value	Pr > F
1	5.75	0.0433
2	0	0.9708
3	2.26	0.1712

The accumulated number of dead individuals was calculated for each cycle, over 78 days (Figure 45).

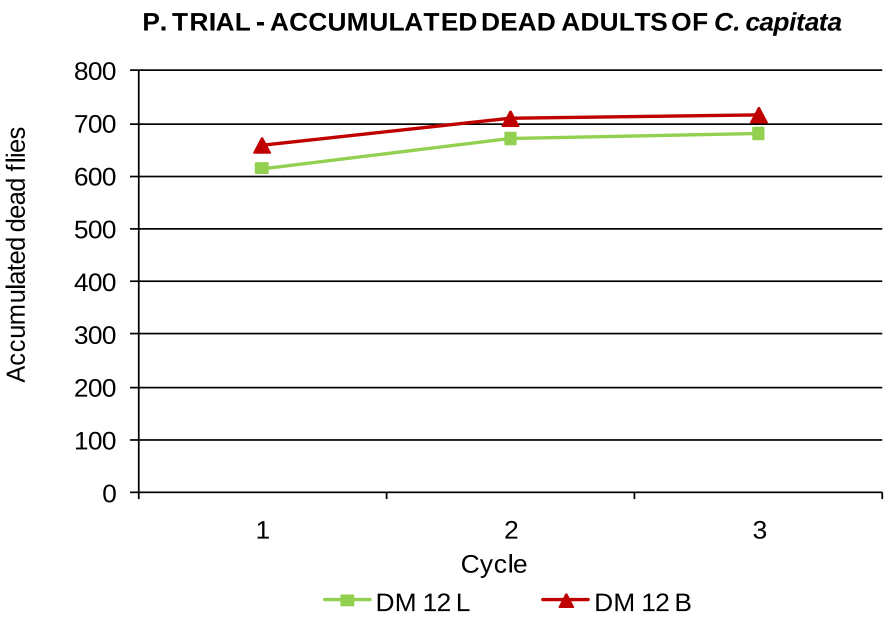


Figure 45. Accumulated captures of medfly in each cycle.

3.4 MASS TRAPPING TRIAL

The total number of *C. capitata* captured in the hundred traps used over the 19 weeks evaluated was 12,574 adults, which is equivalent to 5,515 per ha.

The highest number of captures observed was in the six traps located in the NW extreme of the plot. The three-dimensional representation of the spatial distribution of captures showed that the area with higher catches was close to the hedge in which other medfly hosts were planted (Liquido et al., 1991), including one fig tree, several peach trees and a small vegetable garden with some tomato plants (Figure 46). This chart demonstrated the sum of captures registered over the first six weeks, up to the beginning of harvest. The number of flies accumulated in the traps from the NW corner after the first six weeks was higher than 300 F/T/D.

Initially total captures from all traps were low (Figure 47), but numbers rose quickly in the extreme NW of the plot. Maximum captures (also named picks) registered in a single trap were also included in the analysis and over nine weeks, the highest recorded score was in the trap at the NW extreme of the site. The maximum number of captures/trap/day, 127.29 F/T/D was recorded in the third week (Figure 47).

A chemical spraying was performed at the beginning of October, in order to avoid a pick in the captures as had been registered previously (Figure 47). Immediately after the agrochemical treatment, a second pick was recorded (75 F/T/D) after which, a sudden decrease was registered in mid October that continued below 5 F/T/D throughout to the end of the trial.

After 15 weeks of trial, on 10th December the maximum captures were registered in the trap located in the NE extreme corner, with 0.57 F/T/D recorded. On 31st December with a mean temperature of 14.2ºC, only one individual was found and at the next check, in the first week of 2010, not a single medfly was captured, which signalled the end of the trial.

The long period analyzed in this trial (four and a half months) was characterized by warm weather and summer conditions at the beginning of the trial, with a maximum mean temperature of 25.4ºC and much lower temperatures at the end, when winter conditions brought a minimum mean temperature of 0.3ºC. Rainfall was concentrated in few days, with a maximum registered precipitation of 30 mm (Figure 48).

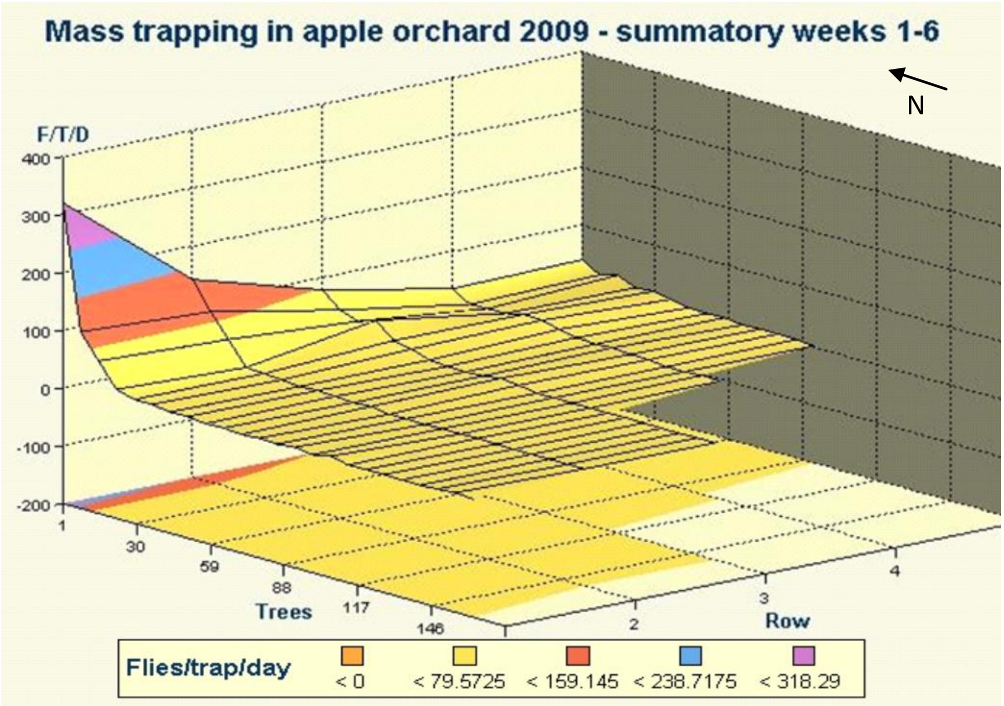


Figure 46. Three-dimensional representation of captures of medfly in the studied plot from the beginning of the trial until the sixth week.

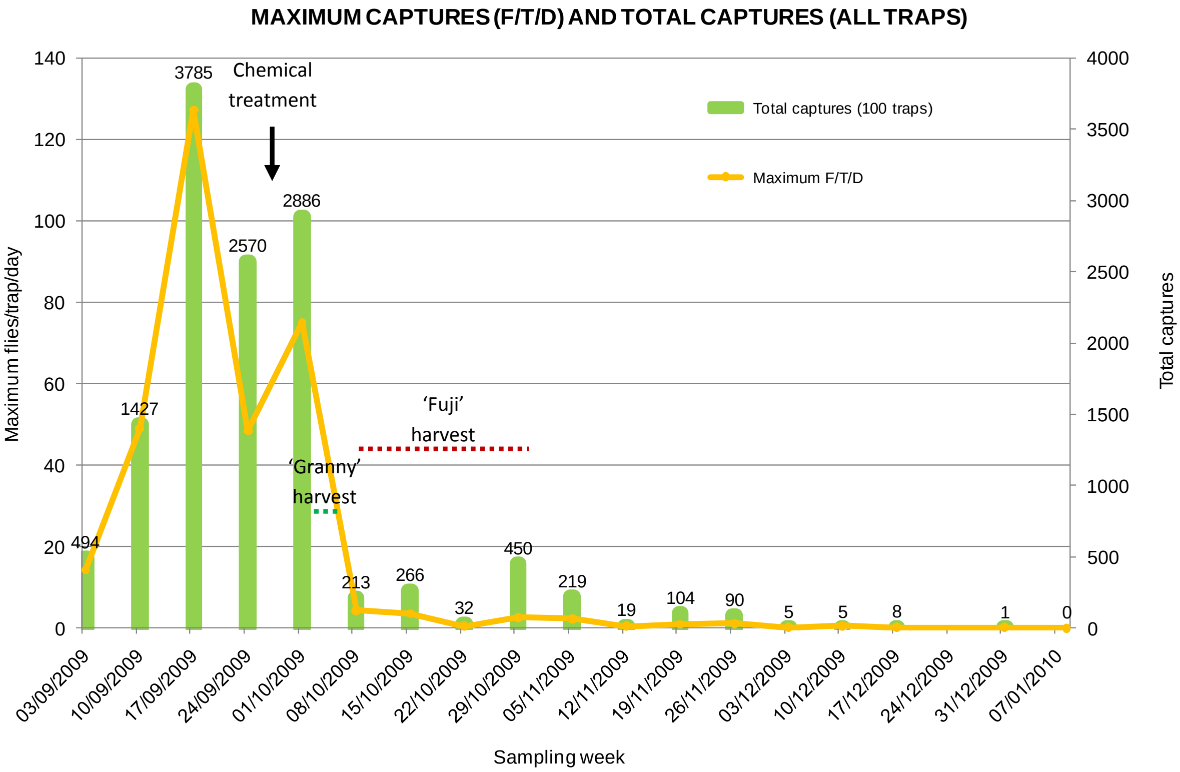


Figure 47. Population dynamics of medfly in a commercial apple plot using mass trapping. The black arrow indicates the only spraying performed against medfly during the studied period and the harvest periods of 'Granny Smith' and 'Fuji' varieties are specified.

The average percentage of females captured was 62.50%, but this figure varied throughout time (Figure 49). After the harvest period, the percentage of males rose, till eventually the proportion of both sexes was identical.

Over the entire period surveyed, live adults registered inside the traps represented only 3% of the total captures.

After an exhaustive examination of 4,000 apples still hanging on trees, no damaged fruit was found in any of the four checks conducted (Table 19).

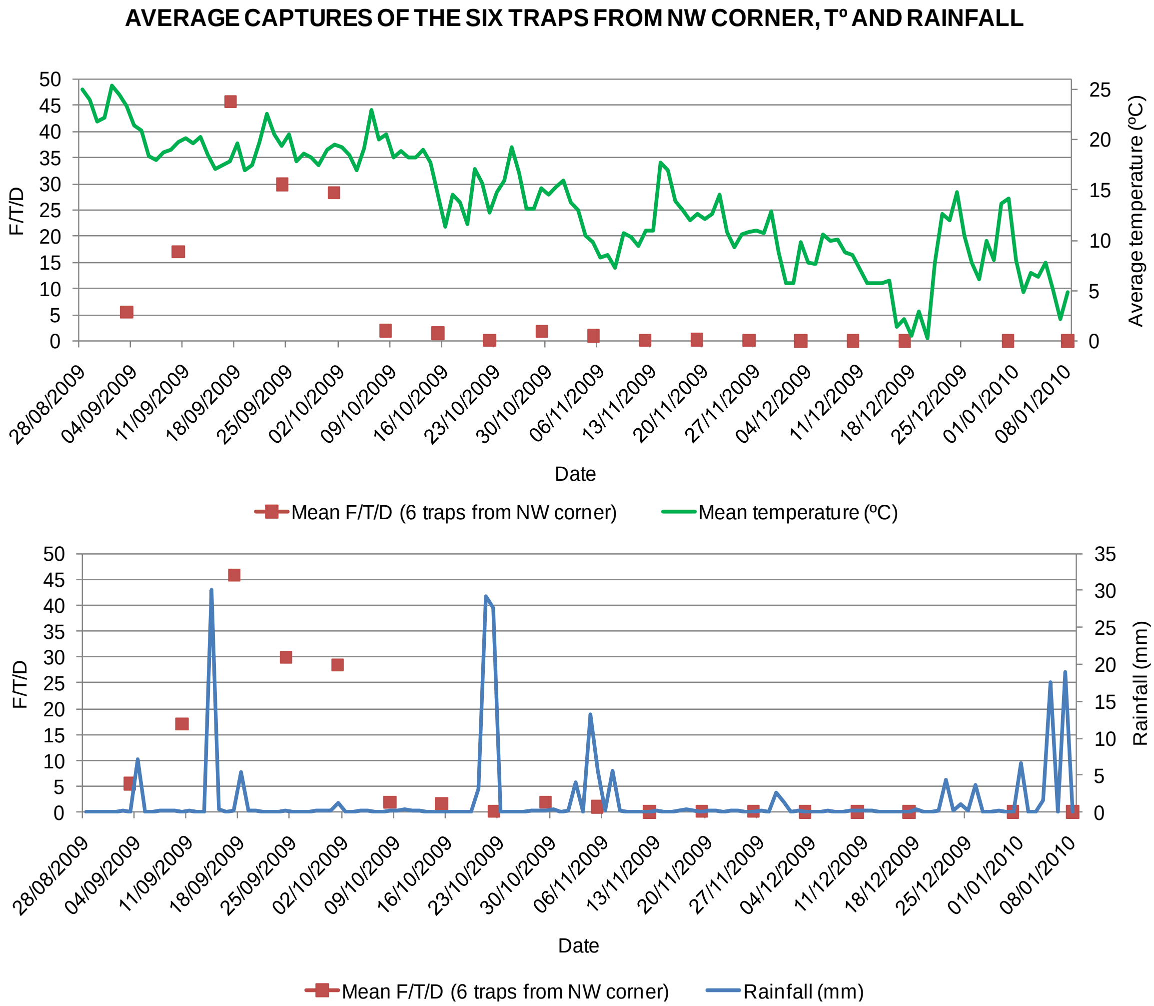


Figure 48. Registration of mean captures of flies/trap/day from the six traps located in the NW extreme, daily mean temperature and daily rainfall over the period of the mass trapping trial.

Once the ‘Granny Smith’ variety was harvested, on 7th October, 2009 and over the twelve following weeks, 1,412 captures were registered, equivalent to 0.17 F/T/D.

The harvest of the ‘Fuji’ variety finished on 2nd November, 2009 and throughout the remaining eight weeks 451 adults were counted, equivalent to 0.08 F/T/D.

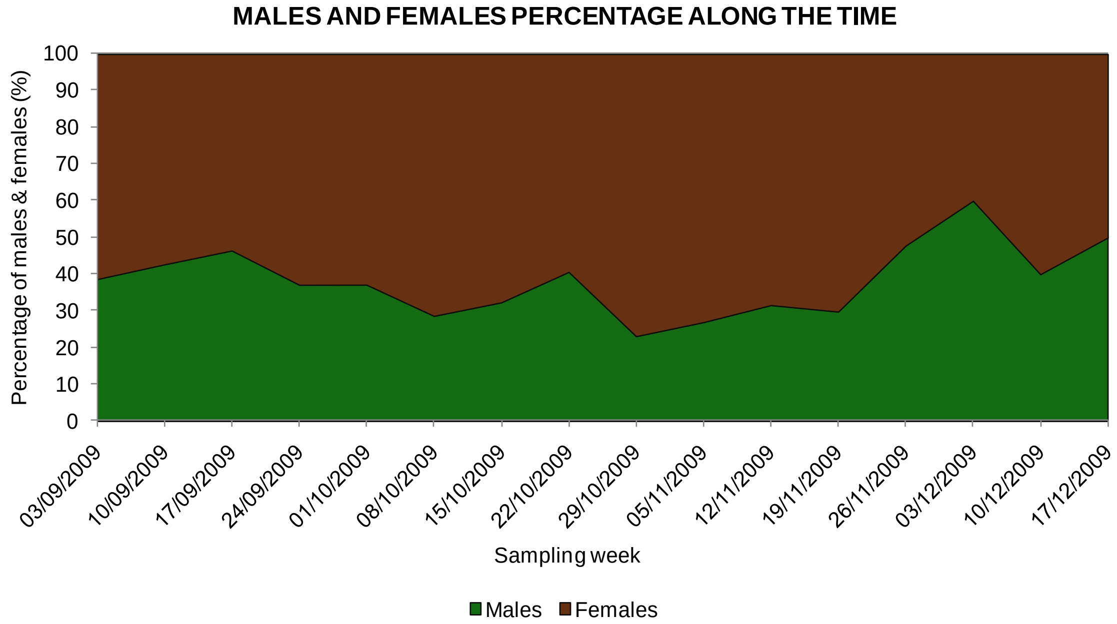


Figure 49. Males and females percentage captured over the full period of the trial.

Table 19. Number of damaged fruits in each evaluation realized in the orchard.

DATE	NUMBER OF EVALUATED FRUITS	NUMBER OF DAMAGED FRUITS
Previous to the trial (2/9/09)	1000	0
10/9/09	1000	0
01/10/09	1000	0
15/10/09	1000	0

4 DISCUSSION

There is a lack of published information on the use of different active ingredients and formulations of insecticides in the mass trapping technique. Despite the need to find a replacement for DDVP for use in fruit fly traps, few papers have been published on this subject. In those publications available, the active ingredient deltamethrin was tested in two Spanish areas using the same methodology as that used in the present studies F. (I) and F. (II): comparative trial with trap rotation (Alemany et al., 2005) (Ros et al., 2005a). The first trial was performed in a citrus orchard on Mallorca Island and the retention systems tested (Scalibur® strips and PermaNet® 75) were reported to be effective for 6 and 12 months respectively and to be good substitutes for DDVP (Alemany et al., 2005). The second trial using deltamethrin as a killing agent was performed in a mango orchard in Malaga. Easy and MTL traps baited with synthetic attractants (ammonium acetate and trimethylamine) plus deltamethrin worked better than with DDVP. These authors supposed a repellent effect on flies approaching the traps baited with DDVP, although further studies must be carried out in order to corroborate this supposition (Ros et al., 2005a) (Ros et al., 2005b). Although in both experiments deltamethrin was used with different formulation, support prototypes and traps, the results agreed with the findings of the present study in respect of the efficacy of this technical insecticide.

One of the control formulations used in the present study was deltamethrin 300 mg and neither the one manufactured on 2007 nor its homologue from the previous year differed in respect of the number of flies found live, which indicates that the product maintained its full effectiveness after one year of storage.

In both formulation tests, the importance of the presentation mode of the insecticide was demonstrated. In F. (I), two formulations with the same dosage of deltamethrin were compared, one in a diffuser (DM 20 B) and the other in the lid impregnated with methylene chloride (DM 20). There were significant differences between them in respect of live and dead flies recorded, the second one achieving better results. In F. (II) the dosage of 2 mg of deltamethrin using the formulation as powder (DM 2) was compared with the same dosage with xylene medium (DM 2 X). They differed in respect of the number of live flies recorded, the first treatment producing better results.

The climatic conditions in which the first two trials were done were ideal for medfly activity because the daily mean temperature varied within the optimum range for this insect, 15 to 30ºC (Duyck et al., 2006) and the highest rainfall registered (22.2 mm on 5th October) did not seem to affect captures in traps. In the position trial, variability in weather conditions could be one of the reasons why the total captures of each cycle varied (1,273, 108 and 17 adults in each cycle).

The fact that no live adults were registered in the position trial could be due to a high level of efficacy of the insecticide used, the active ingredient and formulation that had obtained the best results in earlier trials. The most likely reason, however, could be the length of the cycles which lasted between 21 and 26 days. This would make survival difficult for flies falling into the trap at the beginning of the cycle, although adults falling in a few days prior to the checking date could have been found live.

Trial P. showed differences between positions of insecticides at a very low rate of significance, with higher captures when they were used in the base of the trap. However, in trial F. (I) treatment DM 20 B was composed of a diffuser placed in the base of the trap and achieved significantly the worst results. Data from this trial demonstrated the effectiveness of DM impregnated in the lid while its effectiveness in the other position depended on the kind of presentation used. Movement impregnation was effective but the dispenser was inadequate. Because of the differences in the results obtained from these two trials, further studies will need to be carried out.

The final proof of the efficacy of the formulation insecticide used in the first three studies was its use in a commercial orchard using the mass trapping technique. The current study is the first trial of mass trapping performed using deltamethrin as a replacement for DDVP.

Over the first month and a half, the mean temperature in the survey area was optimal for the development of the pest and, unsurprisingly, high levels of population captures in all traps were registered during the first five weeks of this period. Mean captures in the six traps located in the NW extreme were at a maximum during the period in which the mean temperature was high. Conversely, therefore, cooler weather with minimum temperatures below 10ºC could be one of the causes of the low population captures at the end of the trial. This phenomenon was observed in a comparative trial at field level (Miranda et al., 2001) and it could explain the minimizing of the differences between traps, as observed in another field experiment using mass trapping equipment (Alemany et al., 2005).

Despite the high level of captures, the checking of fruits revealed no damage. Nevertheless, to ensure protection of the harvest, in the fifth week it was decided to undertake a chemical treatment. This could explain the decrease in capture levels in all traps, especially in the one in the NW, which went from a pick of 75 F/T/D to low levels that were maintained below 5 F/T/D until the end of the study. Although there is circumstantial evidence that rainfall affects Tephritid distribution (Vera et al., 2002), during this trial the first rainfall (30 mm) made no difference to the level of captures in any trap. However, 56.8 mm of rainfall over two consecutive days in October coincided with a decrease in captures: totals dropped from 266 adults on the day prior to the rain (October 15th) to only 32 on the checking day after the rainfall (October 22nd). Captures were seen to have recovered to 450 at the following check (October 29th).

In this mass trapping trial the average percentage of females (66.08%) was similar to the average obtained in another trial performed in Girona fruit growing area in 2007 on 'Fuji' variety apple trees and in four other trials on peach trees (65.32%) (See Chapter IV of this Ph.D.). Therefore, the proportion of females using the attractants with three components plus deltamethrin was included in the range of values usually found using this attractant with DDVP.

Analysis of data from all traps throughout the entire study period showed that the trap recording the maximum number of captures was located in the extreme NW of the study area. Here a pick of captured population took place on the third study week (mid September), and registered extremely high scores: 891 flies were captured in only one trap and one week, which equates to 127.29 F/T/D. This pick was twice the maximum value of the studies performed in 2007 which recorded between 50 and 58 F/T/D at the end of August on peach trees from the same area (See Chapter IV of this Ph.D.). The results of this study were also five times higher than picks from other mass trapping trials conducted between 2003 and 2006 in Valencia and Ibiza citrus orchards in mid July and at the end of September, respectively (Martínez-Ferrer et al., 2007).

The number of live adults registered over the entire mass trapping study was 3%, although this value was highly influenced by the level of captures from the 14^{th} week, when 5 individuals were found, one of which was still live (20%). If this incident were not taken into account, the average percentage of live flies would be only 2%, an acceptable level bearing in mind that one of the objectives of the methodology is to cause death to all flies entering the traps.

No damaged apples were found in the four checks made in the last trial. Therefore, mass trapping using deltamethrin competed efficiently with the ripening fruits, in attracting the pest. Although this technique worked reasonably well for the protection of apples, its efficacy fluctuates according to the crop and variety planted. In four trials of mass trapping using DDVP as insecticide in Girona peach plots in 2007 maximum damage recorded was 4.37%, although in four trials on other peach varieties in 2008 in the same area, no damage was found (See Chapter IV of this Ph.D.). Studies of mass trapping using DDPV in peach orchards in Girona province which took place in 2006 showed maximum damage of only 0.69% (Batllori et al., 2008). Citrus orchards planted with early varieties are known to suffer significant medfly damage (Martínez-Ferrer et al., 2007). Trials carried out in the Tarragona area in 1998 using mass trapping with DDVP to protect citrus fruits from medfly, recorded a mere 0.50% damage (Sastre et al., 1999).

Captures were low from the harvesting period until the end of the trial, from 0 to 0.17 F/T/D over the 12 weeks after ‘Granny Smith’ harvest and from 0 to 0.08 F/T/D for the 8 weeks after ‘Fuji’ harvest. Traps were, therefore, very effective in attracting the remainder of the medfly population away from the plot and they also helped to prevent the colonization of nearby plots containing other varieties not yet harvested, such as ‘Pink Lady’ apples (Iglesias et al., 2000).

5 CONCLUSIONS

Trials F. (I) and F. (II) showed that the insecticide deltamethrin at 20 mg dosage impregnated by movement in the lid is a possible substitute for DDVP.

Trial P. revealed a slightly superior effectiveness of the insecticide impregnated by movement in the base of the trap, and trial F. (I) demonstrated that the disposition by movement impregnation in the lid was much more effective than the dispenser.

The prototype of insecticide in a basis of deltamethrin 12 mg used in mass trapping for the control of medfly demonstrated a highly efficient killing action at both low and high population levels, enhancing the death of 98% of flies entering the traps, thus leaving a very low percentage of adults captured live.

This insecticide prototype baited with deltamethrin is a possible substitute for DDVP.

6 REFERENCES

Alemany, A., Miranda, M.A., Vestergaard, F., Abdali, A., 2005. Deltamethrine as a replacement for diclorvos (DDVP) strips used as a retention system in fruit fly traps. Development of improved attractants and their integration into Fruit Fly SIT management programmes, Vienna 5-7 May 2005, pp. 11.

Batllori, L., Escudero, A., Vilajeliu, M., Garcia, F., Benejam, J., 2008. Area-wide mass trapping to control Ceratitis capitata (Wied.) on stone fruits in Girona, NE of Spain. Integrated Plant Protection in Stone Fruit, IOBC/wprs Bulletin 37, 73-82.

DAR, 2010 Agrometeorologia. http://ruralcat.net.

Duyck, P.F., David, P., Quilici, S., 2006. Climatic niche partitioning following successive invasions by fruit flies in La Reunion. Journal of Animal Ecology 75, 518-526.

EEC, 1991. Council Directive of 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC).

Iglesias, I., Carbó, J., Bonany, J., Dalmau, R., Guarter, G., Montserrat, R., Moreno, A., Pagès, J.M., 2000. Pomera, les varietats de més interès.

Liquido, N.J., Shinoda, L.A., Cunningham, R.T., 1991. Host plants of the Mediterranean fruit fly (Diptera: Tephritidae): an annotated world review. Entomological Society of America, Lanham, MD.

Martínez-Ferrer, M.T., Alonso, A., Campos, J.M., Fibla, J.M., Garcia-Marí, F., 2007. Dinámica poblacional de la mosca de la fruta Ceratitis Capitata en tres zonas citrícolas mediterráneas. Levante Agrícola 385, 1-7.

Miranda, M.A., Alonso, R., Alemany, A., 2001. Field evaluation of Medfly (Dipt., Tephritidae) female attractants in a Mediterranean agrosystem (Balearic Islands, Spain). Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 125, 333-339.

Ros, J.P., Castillo, E., Wong, E., Olivero, J., Rubio, J.R., Marquez, A.L., 2005a. Development of traps and killing agents to improve the mass trapping techinique against Ceratitis capitata Wied. and Bactrocera oleae Gmel. (Diptera: Teprhitidae). Development of improved attractants and their integration into Fruit Fly SIT management programmes, Vienna 5-7 May 2005, pp. 27.

Ros, J.P., Wong, E., Olivero, J., Rubio, J.R., Marquez, A.L., Castillo, E., Blas, P., 2005b. Desarrollo de atrayentes y mosqueros para su integración en los programas de trampeo masivo contra la mosca de la fruta (Ceratitis capitata Wied.) y la del olivo (Bactrocera oleae Gmel.). Boletín de Sanidad Vegetal, Plagas 31, 599-609.

Sastre, C., Melo, J.C., Borreli, G., 1999. La captura de hembras: una posible salida en el control de mosca de la fruta (Ceratitis capitata, Wied.) en melocotonero. Phytoma España 113, 42-47.

Vera, M.T., Rodriguez, R., Segura, D.F., Cladera, J.L., Sutherst, R.W., 2002. Potential geographical distribution of the Mediterranean fruit fly, Ceratitis capitata (Diptera : Tephritidae), with emphasis on Argentina and Australia. Environmental Entomology 31, 1009-1022.

6. CHAPTER IV: Mass trapping control technique for *Ceratitis capitata* in peaches

INDEX

1 INTRODUCTION 159

2 MATERIAL AND METHODS 161

2.1 EXPERIMENT 1. COLONIZATION PROCESS AND SPATIAL DISTRIBUTION 161

2.1.1 EXPERIMENTAL PLOTS 161

2.1.2 TRAPPING EQUIPMENT 163

2.1.3 EXPERIMENTAL SET UP 163

2.1.4 STATISTICAL ANALYSIS 164

2.2 EXPERIMENT 2. LEVEL OF PROTECTION PROVIDED FOR THE FRUIT BY THE MASS TRAPPING TECHNIQUE AND INSECTICIDES 164

2.2.1 EXPERIMENTAL PLOTS 164

2.2.2 TRAPPING EQUIPMENT 166

2.2.3 EXPERIMENTAL SET UP 166

2.2.4 STATISTICAL ANALYSIS 167

2.3 EXPERIMENT 3. PROPORTION OF TRAPS TO BE CHECKED 167

2.3.1 EXPERIMENTAL SET UP 167

2.3.2 STATISTICAL ANALYSIS 168

3 RESULTS 168

3.1 EXPERIMENT 1. COLONIZATION PROCESS AND SPATIAL DISTRIBUTION 168

3.1.1 POPULATION DENSITY CAPTURED IN TRAPS 168

3.1.2 DAMAGE LEVEL 170

3.1.3 COLONIZATION PROCESS AND SPATIAL DISTRIBUTION 170

3.2 EXPERIMENT 2. LEVEL OF PROTECTION PROVIDED FOR THE FRUIT BY THE MASS TRAPPING TECHNIQUE AND INSECTICIDES 173

3.2.1 POPULATION DENSITY CAPTURED IN TRAPS 173

3.2.2 DAMAGE LEVEL 173

3.3 EXPERIMENT 3. PROPORTION OF TRAPS TO BE CHECKED 173

4 DISCUSSION 178

4.1 EXPERIMENT 1. COLONIZATION PROCESS AND SPATIAL DISTRIBUTION 178

4.2 EXPERIMENT 2. LEVEL OF PROTECTION PROVIDED FOR THE FRUIT BY THE MASS TRAPPING TECHNIQUE AND INSECTICIDES 185

4.3 EXPERIMENT 3. PROPORTION OF TRAPS TO BE CHECKED 186

5 CONCLUSIONS 186

6 REFERENCES 187

1 INTRODUCTION

Peach fruit is a host included in the heavily or generally infested hosts list (Weems, 1981) and in a study of the host-specific demography of *Ceratitis capitata* (Wiedemann) it was found to have a survival rate from larvae to adult of 65%, the third highest of the twenty host species analyzed (Carey, 1984). In the stone fruit area of Girona its population has been shown to be increasing since the 90's, with high levels in 2001 and 2003, low levels in 2005, moderately high in 2006 and high in 2007 and 2008 (Batllori et al., 2008) (Escudero-Colomar et al., 2008) (Escudero-Colomar et al., 2009). Relatively low levels of medfly population were registered in 2009 and very low levels in 2010.

In 1999, cooperatives from Girona area agreed to adopt the principles of integrated fruit production (Batllori et al., 2003) and as part of this methodology, growers have recently begun to use a mass trapping system for fruit flies, adding the necessary insecticide applications only when the population level requires them.

The *Royal Decree* 461/2004 (BOE, 2004) established compulsory control measures against *C. capitata* all over Spain. The prevention and control programme in Catalonia is based on the early installation of the mass trapping technique against adults using suitable traps and lures, the destruction of all non commercial fruits left on trees and on the ground, and the obligation to remove abandoned host plantations (DOGC, 2004). Nowadays, the compulsory control programme against this species is being implemented in all stone fruit orchards in Girona fruit growing area (Batllori et al., 2008).

Over the last decade, the efficiency of the mass trapping technique used against *C. capitata* has been confirmed by researchers in other countries and on several different crops including apple trees in Spain (Batllori et al., 2003) (Batllori et al., 2005) (Escudero et al., 2005), citrus orchards in Israel (Nestel et al., 2004), Spain (Alemany et al., 2004) (Alonso-Muñoz and García-Marí, 2004) (Fibla et al., 2007) (Campos et al., 2008) and Portugal (Cabrita and Ribeiro, 2006), grapes in Spain (Lucas and Hermosilla, 2008), grapefruit in Israel (Nestel et al., 2004) and peach trees in Spain (Sastre et al., 1999) (Batllori et al., 2008).

Another Tephritid species like *Bactrocera oleae* (Gmelin) have been controlled by mass trapping system on olive trees in Greece (Broumas et al., 2002).

Fly movement patterns and spatial dynamics must be considered when designing an area-wide management strategy against *C. capitata* and therefore in an IPM program in an agricultural landscape, spatiotemporal analysis is required (Aluja, 1993) (Papadopoulos et al., 2003) (Nestel et al., 2004) (Israely et al., 2005) (Trematerra et al., 2008) (Sciarretta et al., 2009). Temporal and spatial dynamic information at micro-level (inside the orchard) is important when deciding control measures for certain parts of the plot, and brings corresponding time and cost savings (Papadopoulos et al., 2003). Male and female movement patterns must be studied separately in order to discover the effect that gender might have on fly short and long range displacements (Aluja, 1993).

It is helpful to group the parameters for judging the effectiveness of the mass trapping method under headings such as material (trap type, attractant formulation, insecticide, trap density, etc.), biological factors (pest population density, tree size, fruit variety, etc.), climatic factors (t, R.H., rainfall, winds, etc.), cultural factors (irrigation, destruction of non commercial fruits, etc.) and other parameters (number of years the method has been applied in the same orchard, etc.) (Broumas et al., 2002).

During the period in which the young fruit is ripening, pest control advisers must periodically check all or a representative proportion of the traps, in order to estimate the population level in the orchard and to decide if and when an insecticide treatment is necessary. For the effective design of mass trapping strategy, it is important to optimize the time spent checking traps in order to reduce production costs.

Because of the potential economic impact of the pest on the peach crop, the main objective of this study was to optimize the application of mass trapping technique for *C. capitata.*

The specific aims were:

1. To describe the pest colonization process at orchard level including movement patterns throughout time and the gender proportion dynamics in peach orchards. This study would take into account parameters such as the orchard location and size, the sensitivity of the fruit species, the plant species inside and surrounding the orchard and the application of insecticides.

2. To test the level of fruit protection in peach orchards of the Girona province where mass trapping methodology is used.

3. To determine the minimum proportion of traps that must be checked to ensure a reliable estimate of the captured population.

2 MATERIAL AND METHODS

2.1 EXPERIMENT 1. COLONIZATION PROCESS AND SPATIAL DISTRIBUTION

2.1.1 Experimental plots

The research was conducted during 2007 in four plots whose sizes ranged from 0.72 to 2.06 ha (Table 20 and Figures 50 to 52). These plots were situated in commercial orchards of peach trees, one with the 'Early O'Henry' variety, and the other three with the 'Merryl O'Henry' variety.

Mass trapping was used in all four orchards to control the population of the species *C. capitata*.

Data gathered over several years shows that the average harvest period for 'Merryl O'Henry' peaches grown in the Girona fruit growing area varies from August, 2nd to August, 15th (Carbó et al., 2002). The 'Early O'Henry' variety is harvested between 7 and 8 days before 'Merryl O'Henry', taking into account data from several years (Carbó et al., 2002), although in the studied plot A1 ('Early O'Henry') the harvest occurred 14 days earlier than in plot A2 ('Merryl O'Henry').

Table 20. Description of orchards from experiment 1: plot identification, size, variety, plantation frame (distance among trees), plantation year, height above sea level and distance to the sea.

PLOT	SURFACE (ha)	VARIETY	PLANT. FRAME (m)	PLANT. YEAR	ALTITUDE (m)	DISTANCE TO THE SEA (km)
A1	2.06	'Early O'H.'	5 x 4	1998	1	1.00
A2	1.93	'Merryl O'H.'	5 x 4	1998	1	1.00
B	0.85	'Merryl O'H.'	5 x 2	2000	0	5.14
C	0.72	'Merryl O'H.'	5 x 2	2000	0	2.71

Plots A1 and A2 were located close together in the same orchard, in Alt Empordà County. The surrounding area was home to a large 'Early Red One' apple orchard to the North border, a 'Summer Lady' peach orchard to the West, a 'Red Gem' peach plantation and reeds to the South and a road to the East.

Plot B (Baix Empordà County) was limited by irrigation channels to the North-West and South-West, a fig tree, *Ficus carica* Linnaeus to the West corner, a 'Fuji' apple plantation to the South-East and a 'Conference' pear orchard to the North-East.

Plot C (Baix Empordà County) was surrounded by a heterogeneous cluster of species (cypresses, *Cupressus sempervirens* Linnaeus, walnut trees, *Juglans* *sp*., willow, *Salix sp*., salt cedar, *Tamarix sp*. and blackberries, *Rubus sp*.) with an abandoned peach orchard to the North, a peach plantation to the West, reeds to the South and an apple plot to the East border.

Figures 50, 51 and 52. Aerial view of plots A1, A2, B and C.

During the experimental period, meteorological data were collected at two stations (DAR, 2008), one located in the same area of plots A1 and A2, and the other close to plots B and C. Both stations registered almost exactly the same temperatures and rainfall. Figure 53 shows the average, minimum and maximum temperature and the relative humidity registered in Baix Empordà area.

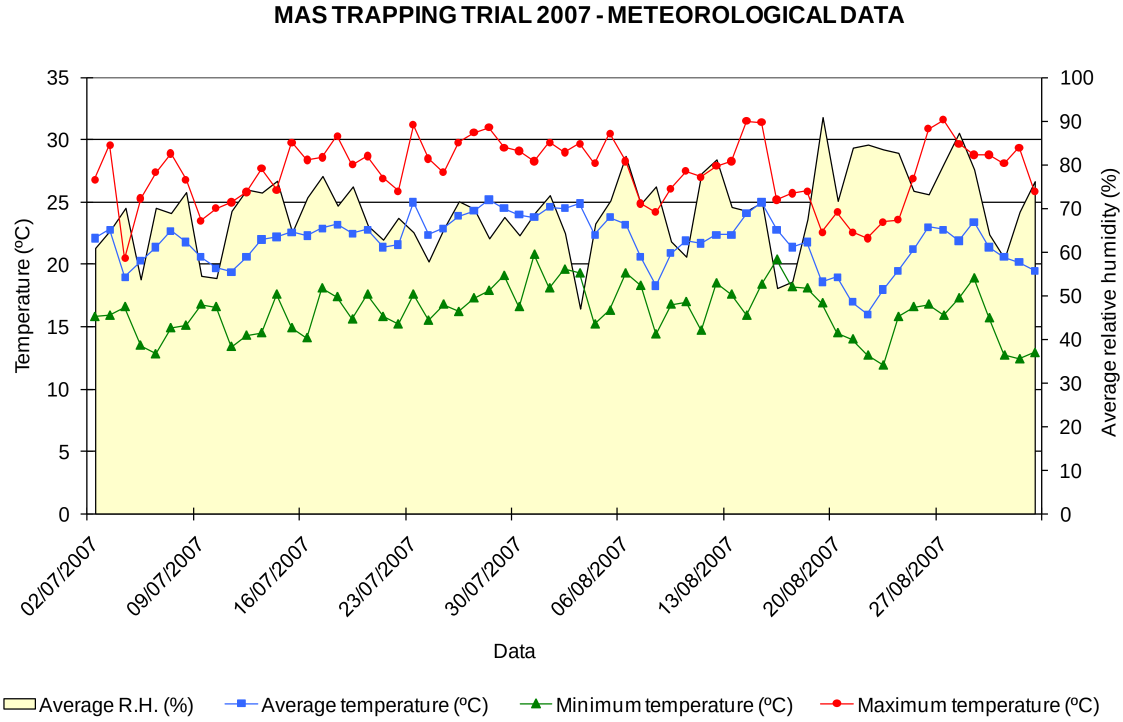


Figure 53. Daily data for several climate variables, average air temperature and relative humidity recorded during the survey period at the meteorological station located in the same area than B and C orchards.

2.1.2 Trapping equipment

Maxitrap® traps (Probodelt S.L., Amposta, Spain) were used. These have been proved to be some of the most effective of those available in the market (Alonso-Muñoz and García-Marí, 2007) (Lucas and Hermosilla, 2008) (Navarro-Llopis et al., 2008).

Traps were baited with the dry food-based synthetic attractants ammonium acetate, diaminoalkane and the coadyuvant trimethylamine incorporated in three slow release membrane diffusers, Ferag® CC 3D TM (SEDQ S.L., Barcelona, Spain). The volatile insecticide placed inside the traps was Ferag® ID TM, its active ingredient being dichlorvos 400 mg (SEDQ S.L.).

The same materials (trap, attractants and insecticide) were described and used in the monitoring study performed in Girona fruit growing area during 2005, 2006 and 2007 with high levels of efficacy (Escudero-Colomar et al., 2008).

2.1.3 Experimental set up

Traps were installed following a homogeneous distribution pattern. The density of traps used for *C. capitata* mass trapping is 1 trap/200 m^2, which is equivalent to 50 traps/ha. Nevertheless, in the present study the density of traps was adapted to the irregular shape of each plot and the real density used ranged from 46 to 64 traps/ha.

Traps were placed inside the tree canopy, in the South-East part of the trees, at a height of 1.5 m. They were installed in plots A1 and A2 on 14th June and in plots B and C on 21st June, 2007.

In order to avoid the emigration of *C. capitata* adults to surrounding orchards containing later varieties, traps were left in the trees for a minimum of 15 days after harvest (which took place in the first fortnight of August), until the non commercial fruit had been destroyed. This has been the practice for some years in Girona province (Batllori et al., 2008).

Traps were spatially identified to allow tracking of the developing pattern of captures. All traps were checked weekly, and all captures were quantified and sexed over a period of nine weeks, from July, 3rd to August, 28th. Data were recorded and transposed to show number of flies/trap/day (F/T/D), males/trap/day, females/trap/day and total captures/ha, in order to be able to compare the quantity of flies in different plots.

In the few weeks before and also during harvest, damage evaluation was carried out by checking the presence of lay symptoms or nutritional rot in 500 fruits/ha of those still hanging on the trees, without picking them.

All plots studied were situated in commercial orchards, and because of the high population level found, it proved necessary to spray chemicals as reinforcement to the main control technique. During the survey period, plots A1, A2 and B were sprayed once with Malathion 50% and again with Trichlorfon 80% but plot C was sprayed only once with Trichlorfon 80%.

When necessary, chemicals with little or no effect on the target species were applied against other pests such as *Anarsia lineatella* Zeller, *Grapholita molesta* (Busck), *Myzus persicae* (Sulzer) and diseases such as *Taphrina deformans* (Berk) and *Monilia fructigena* Honey.

2.1.4 Statistical analysis

A statistical analysis of the influence of the factors plot and date and their interaction with analysed variables: females/trap/day and males/trap/day was carried out through GLM procedure of Enterprise Guide analysis of the SAS program. To meet the ANOVA assumptions a logarithmic transformation of variables was performed. Means were separated by Tukey's studentized test of Enterprise Guide analyse of the SAS program.

A statistical analysis of differences between the number of males and females captured in each peach plot over the period in question was carried out using ANOVA, one-way analysis of Variance, and Tukey's studentized test.

Spatial-distribution graphs (three-dimensional graphs of surface) were produced using Enterprise Guide analysis of the SAS program.

2.2 EXPERIMENT 2. LEVEL OF PROTECTION PROVIDED FOR THE FRUIT BY THE MASS TRAPPING TECHNIQUE AND INSECTICIDES

2.2.1 Experimental plots

The research was conducted during 2008 in four plots ranging in size from 0.30 to 1.06 ha (Table 21 and Figures 54 to 56). As in the previous year, all plots were located in commercial peach orchards. Three plots were planted with the 'Elegant Lady' variety and the other with 'Symphonie' and 'Elegant Lady' cultivars.

Mass trapping was used in all four orchards to control the population of the species *C. capitata*.

In the Girona fruit growing area, the average harvest period for both varieties is between July, 23rd and 24th to August, 3rd, respectively (Carbó et al., 2002). In plots E1 and E2, harvest began on 1st August, in plot F on 23rd July, in plot G ('Elegant Lady') on 24th July and in plot G ('Symphonie') on 30th July, 2008.

Table 21. Description of each orchard from experiment 2: plot identification, size, variety, plantation frame (distance among trees), plantation year, height above sea level and distance to the sea.

PLOT	SURFACE (ha)	VARIETY	PLANT. FRAME (m)	PLANT. YEAR	ALTITUDE (m)	DISTANCE TO THE SEA (km)
E1	0.91	'Elegant Lady'	5.4 x 3.7	2000	5	6.70
E2	1.06	'Elegant Lady'	5.4 x 3.7	2000	5	6.90
F	0.42	'Elegant Lady'	5 x 3	1998	4	1.75
G	0.30	'Symphonie' & 'Elegant Lady'	5 x 2.5	2002	5	4.40

Plots E1 and E2 were located in the same orchard in Baix Empordà County and were separated by a track. The surrounding area was home to sunflower orchards to the North of both plots, the South of E1, and adjacent to the West border. The South of E2 was bordered by a 'Rich Lady' peach orchard and the Eastern border by a plant barrier composed of several cypresses, one mulberry tree, *Morus* sp. and a fig.

Plot F was located in Alt Empordà County and was bordered by cypresses and houses to the North, by a 'Big top' peach orchard to the East and South and by a 'Merryl O'Henry' peach to the West.

Plot G was located also in Alt Empordà County and was limited by a road and alfalfa, *Medicago sativa* Linnaeus to the North, alfalfa to the East, the same variety of peaches to the South and a field left in fallow to the West border.

Figures 54, 55 and 56. Aerial view of plots E1 and E2.

During the ripening period, meteorological data were collected at weather stations (DAR, 2008) located in both areas (Alt and Baix Empordà), each recording similar temperatures and precipitation levels. Figure 57 shows the data corresponding to the parameters temperature and relative humidity in Baix Empordà.

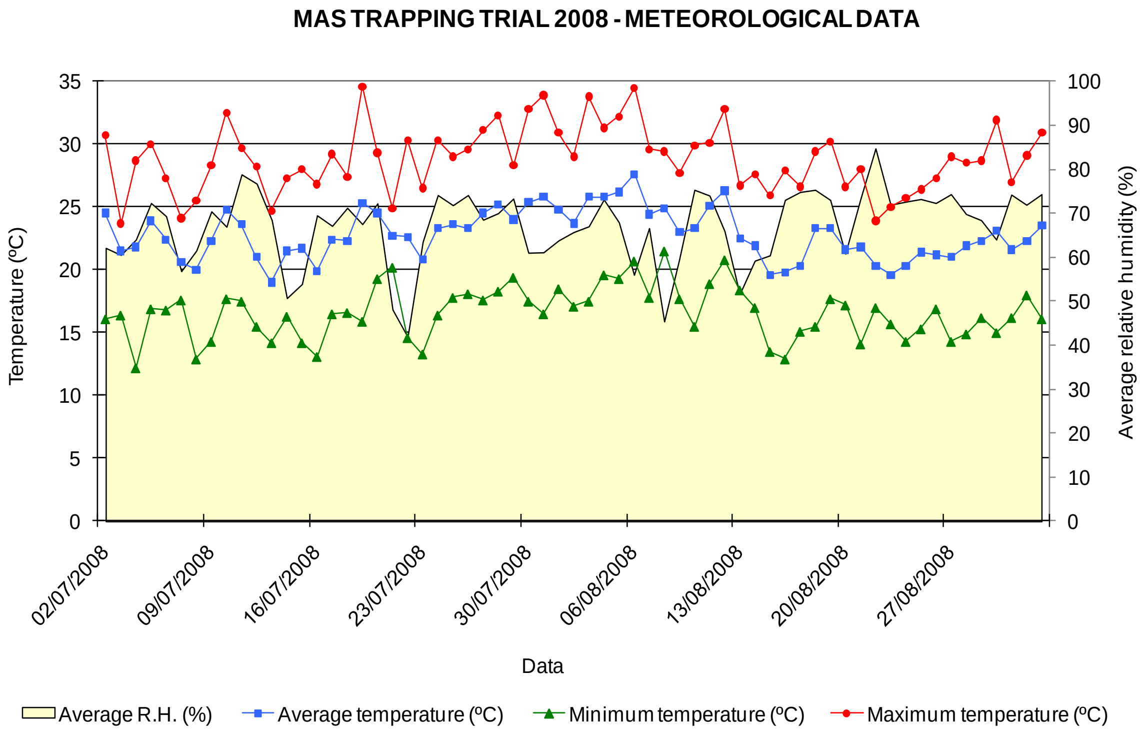


Figure 57. Daily data for several climate variables, average air temperature and relative humidity recorded during the survey period at the meteorological station located in the same area than E1 and E2 orchards.

2.2.2 Trapping equipment

In this study Maxitrap® traps were used. They were baited with the dry food-based synthetic attractants ammonium acetate, trimethylamine and diaminoalkane incorporated into a unique slow release membrane diffuser using the commercial formulation named Ferag® CC D TM (SEDQ, S.L.). The difference between the lures utilized last year and this is the form of presentation of the three compounds, in this case in a unique dispenser that allows an easier and quicker manipulation of the product whilst maintaining the same level of efficacy. The insecticide utilized was the same as that used in the previous study, Ferag® ID TM which has dichlorvos 400 mg as its active ingredient.

2.2.3 Experimental set up

Between 30 and 55 traps/plot were installed following a homogeneous distribution pattern. They corresponded to 52 and 55 traps/ha in plots E1 and E2, 90 traps/ha in plot F, because of the irregular shape of the orchard and 100 traps/ha in plot G, because of the population level of the previous years. All traps were placed in the South-Eastern side of the tree canopy, at a height of 1.5 m.

Trapping equipment was installed in plots E1 and E2 on 17th June, in plot F on 30th June, and in plot G on 25th June, 2008.

All traps were serviced during a single day: in plots E1 and E2 on 28th July, in plot F on 22nd July, and in plot G on 25th July. Traps were hung in the trees between 22 and 41 days during the ripening period of the peaches.

Traps were spatially identified and captures were reviewed, quantified and sexed in order to ascertain their spatial distribution at plot level. Data were recorded and transposed to show the number of flies/trap/day (F/T/D), males/trap/day, females/trap/day and total captures/ha, in order to be able to compare the quantity of flies in different plots.

During the checking days, a damage evaluation was carried out by checking the presence of lay symptoms (caused by the laying of eggs) or nutritional rot in 500 fruits/ha still hanging on the trees, without picking them.

During 2008, the population of *C. capitata* in peaches in the Girona fruit growing area was higher than the previous year (Escudero-Colomar et al., 2009). Because of this high level, a chemical treatment was used to reinforce the mass trapping technique when the captures level exceeded five females/trap/day (Batllori et al., 2008). During the ripening period, one spray was applied to plots E1 and E2 using Methyl chlorpyrifos 22.4% and another with Trichlorfon 80%. None chemical treatment against medfly was necessary in plot F but plot G was sprayed three times, the first time with Phosmet 50% and the rest with Malathion 50%. As in the trial from the previous year, when necessary, chemicals with little or no effect on the target species were applied against other pests such as *G. molesta*, *M. persicae* and *Quadraspidiotus perniciosus* (Comstock) and also against diseases such as *T. deformans*, *M. fructigena* and powdery mildew, *Sphaerotheca pannosa* (Wallr).

2.2.4 Statistical analysis

Spatial-distribution graphs, specifically three-dimensional charts of surface with its corresponding projection in two dimensions were produced with Enterprise Guide analysis of the SAS program.

2.3 EXPERIMENT 3. PROPORTION OF TRAPS TO BE CHECKED

2.3.1 Experimental set up

This study was performed with the same data (flies/trap/week) obtained in the first experiment of this chapter and therefore, the experimental plots, trapping material and experimental set up corresponds to the one described above.

2.3.2 Statistical analysis

To calculate the minimum proportion of traps to be checked, the average number of captures that would have been observed checking subsets of traps was estimated. Each subset of traps comprised between 20 and 90% of the total number of traps, at a 5% interval. For each week and subset size, 5,000 simulations were carried out, selecting randomly the traps took into account in each simulation. Each simulated mean number of captures per trap and week was compared to the mean number of captures per trap and week observed from all the traps ± 10%. A simulated mean was assumed to be different from the observed one when it did not fall within this interval. The error associated with each subset of traps was the percentage of times that the simulated mean fell outside the interval. In order to avoid high level of damage produced by a high population level, error values were set at 10 and 25%. A macro written in IML, matrix language from the Statistical Analyse Program SAS, was devised to make the calculations.

In order to ascertain an appropriate reduction in the proportion of traps to be checked in both the periphery and the inner part of the plot, the analysis for each group of traps was repeated for those located in the periphery (traps hung along the perimeter) and those located in the inner area.

3 RESULTS

3.1 EXPERIMENT 1. COLONIZATION PROCESS AND SPATIAL DISTRIBUTION

3.1.1 Population density captured in traps

The number of captures of *C. capitata* registered in each of the four peach plots ranged between 4,012 and 30,577 individuals, with a total of 46,052 captures.

The proportion of females was higher than that of males, ranging from 60 to 73%, with an average for the four plots of 65% females.

Applying the GLM procedure of SAS to data collected on females/trap/day and males/trap/day, statistical differences according to the date, the plot and the interaction were found (Table 22). That means that in each plot catches of medfly have followed a different pattern over the time. The R^2 coefficient calculated for the parameter females/trap/day showed a better fit (0.6738) than males/trap/day (0.5964). The curves found with linear models for each plot using females/trap/day and males/trap/day showed that the slopes were different and therefore the curves intersect, being the interaction of the factors qualitative (Figure 58). As a consequence, these factors should be read in conjunction and not separately.

Table 22. Statistical signification taking into account α = 0.05 (signification level of 5%) for analysed variables: females/trap/day and males/trap/day.

EFECT	NUM DF	F-VALUE (♀)	Pr > F (♀)	F-VALUE (♂)	Pr > F (♂)
Model	7	805.23	<.0001	575.79	<.0001
Date	1	3444.46	<.0001	2264.62	<.0001
Plot	3	347.89	<.0001	226.99	<.0001
Plot*Date	24	382.83	<.0001	361.64	<.0001

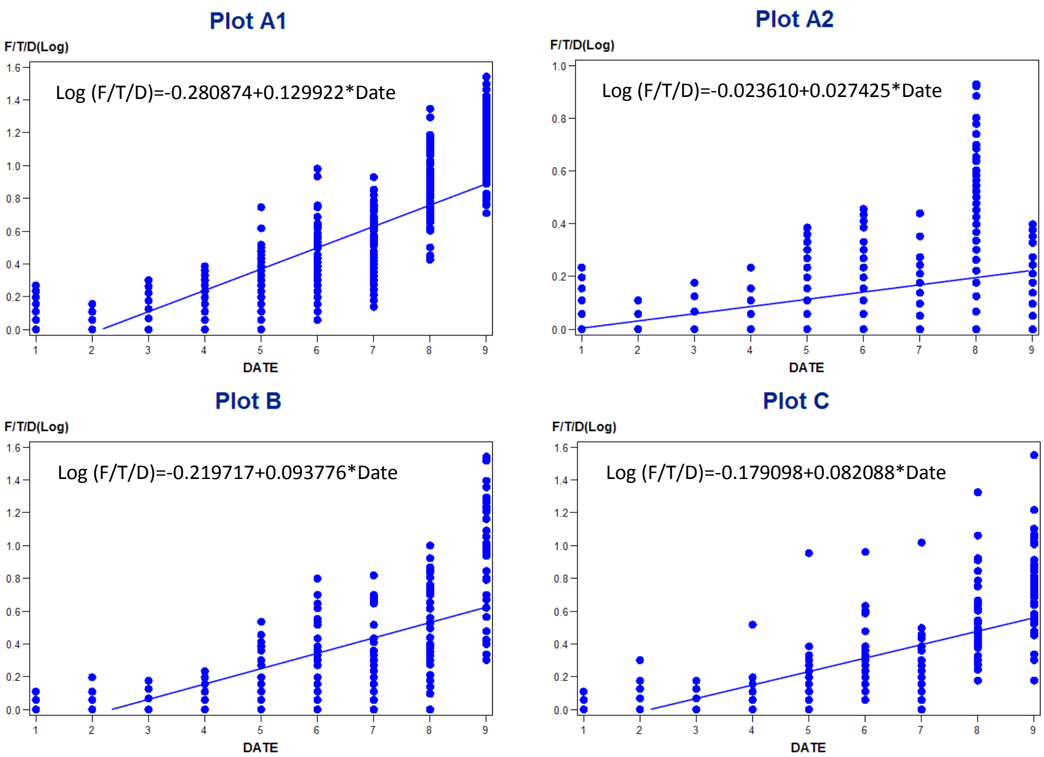


Figure 58. Graphs of the regression curves obtained with the GLM procedures for the variable female/trap/day over time for each plot. The equation is showed in each graph.

The sexes proportion using male:female/trap/day parameters was calculated and the variance was analysed by plot (Table 23). For plot A1 there was a low ratio male:female/trap/day until the 6th week. During the 7th week, males and females were at the same level and there was a high ratio during the last fortnight. For plot A2 the ratio was low until 7th week and during the last fortnight it was recorded the same level of captures for both sexes.

Plot B registered a low ratio until 7th week but during the last fortnight, the ratio was higher than 0.5, thus confirming a higher proportion of males.

Plot C had a low ratio until 7th week. During the following week, levels were similar and during the last week (early September) the ratio was higher than

0.5. At the end of the study period, therefore, in three out of four cases, the ratio of males to females was much higher than 0.5 (Table 23).

Table 23. Average of the ratio male:female/trap/day (M/F), standard deviation and significance level for every week of survey and for each plot. Values followed by the same letter in the same column are not significantly different (Tukey's studentized test, $P < 0.05$) n = 98, 125, 39 and 42, respectively.

PLOT							
A1		**A2**		**B**		**C**	
Week	Ratio M/F	Week	Ratio M/F	Week	Ratio M/F	Week	Ratio M/F
9	0.82 ± 0.22 a	9	0.52 ± 0.56 a	9	0.89 ± 0.40 a	9	0.73 ± 0.38 a
8	0.69 ± 0.34 b	8	0.49 ± 0.37 a	8	0.60 ± 0.41 b	8	0.52 ± 0.27 b
7	0.52 ± 0.34 c	7	0.24 ± 0.39 b	7	0.29 ± 0.32 c	7	0.15 ± 0.23 c
6	0.24 ± 0.22 d	4	0.15 ± 0.38 cb	4	0.10 ± 0.25 d	6	0.13 ± 0.25 c
5	0.12 ± 0.19 e	6	0.15 ± 0.25 cb	5	0.08 ± 0.20 d	3	0.12 ± 0.44 c
1	0.09 ± 0.29 e	1	0.11 ± 0.31 cb	2	0.05 ± 0.22 d	4	0.08 ± 0.26 c
4	0.09 ± 0.20 e	3	0.11 ± 0.56 cb	6	0.04 ± 0.08 d	5	0.07 ± 0.22 c
3	0.04 ± 0.19 e	5	0.09 ± 0.46 cb	1	0.03 ± 0.16 d	2	0.05 ± 0.22 c
2	0.03 ± 0.18 e	2	0.06 ± 0.28 c	3	0.00 ± 0.00 d	1	0.02 ± 0.11 c

3.1.2 Damage level

Although chemical control was used as reinforcement to the mass trapping methodology in all plots studied, in three cases the registered damage level (Table 24) was higher than economically tolerable for fruit growers (1%).

Table 24. Total number of individuals of *C. capitata* captured in traps, total captures per hectare and percentage of damage registered during the harvest week.

PLOT	TOTAL CAPTURES	CAPTURES / ha	% DAMAGE
A1	30,577	14,843	4.37
A2	4,012	2,079	1.89
B	6,535	7,688	2.80
C	4,928	6,844	0.83

3.1.3 Colonization process and spatial distribution

Orchards A1 and A2 were of similar size and were located close to each other, separated by a waste channel colonized by plants. In both plots, captures began at the separation edge (Figure 59) and during the first four weeks, population levels captured were alike. The colonization process was observed in both plots from the common border to the rest of the surface. From the fifth week, the capture level rose in the earlier variety, 'Early O'Henry', and maintained this trend until the end of the trial.

During the survey period, a temporal and spatial heterogeneity was observed in captures in both orchards. When three-quarters of the fruit harvest was completed in A1 (during the 8th week), A2 captures rose by a multiple of six when compared with the previous week. Eventually, during the 9th and last week of study, when two-thirds of the fruit from A2 had been harvested, the average capture level (F/T/D) in this plot was 39 times lower than that in A1. At that point, the pest population on A2 fell by a multiple of seven, when compared with the previous week (the 8th one).

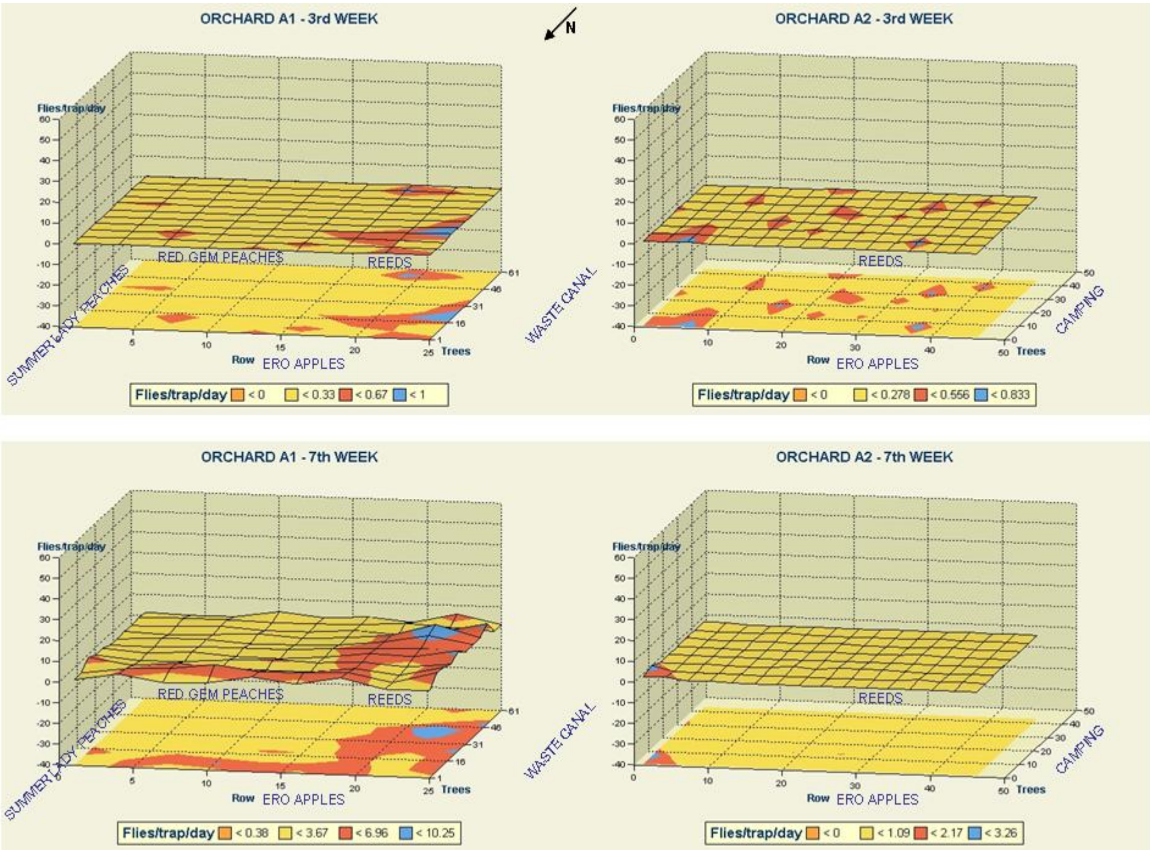


Figure 59. Graphs of plots A1 and A2 showing the spatial distribution and change of captures/trap/day over time (3rd and 7th week of study). The surrounding plots and vegetation are shown with blue letters.

In plot B, the first captures were localized to the border adjacent to the ‘Fuji’ apple orchard but from the second week until the end of the survey, the maximum population was always registered in the South-Western corner, close to the irrigation channel and the fig tree. Figure 60 shows the spatial distribution of captures in the 5th and 9th weeks monitored. These graphs show that maximum captures were located in both weeks in the same area, varying from 2 to 58 F/T/D.

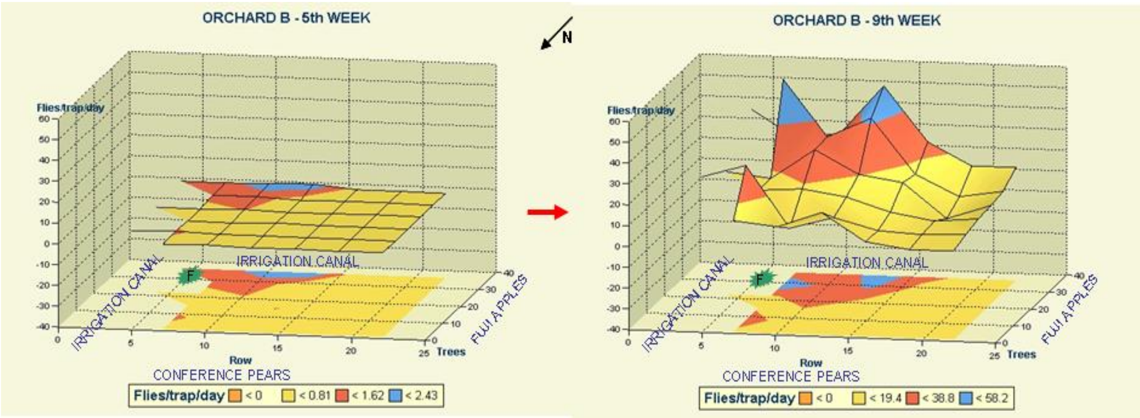


Figure 60. Spatial graphs of plot B showing the number of flies/trap/day captured during the 5th and 9th week. The surrounding plots and vegetation are shown with blue letters. The fig tree has been identified as a green symbol and the letter F.

In orchard C (Figure 61), from the first week and throughout the survey, higher captures were located in one corner, close to the Northern hedge which contained a range of trees and shrubs. The area around plot C was examined to identify available hosts or non-host species: blackberry, willow, walnut, cypress and salt cedar.

Approximately 100 g of blackberries were collected and left to decompose in the chamber at 25ºC, and records were made of any observed larvae of *C. capitata*.

Behind the border associated with higher captures (North), there was an abandoned plot containing peach trees with some hanging fruit. During the last week of the study it was observed that 21% of fruits from this plot had larval damage.

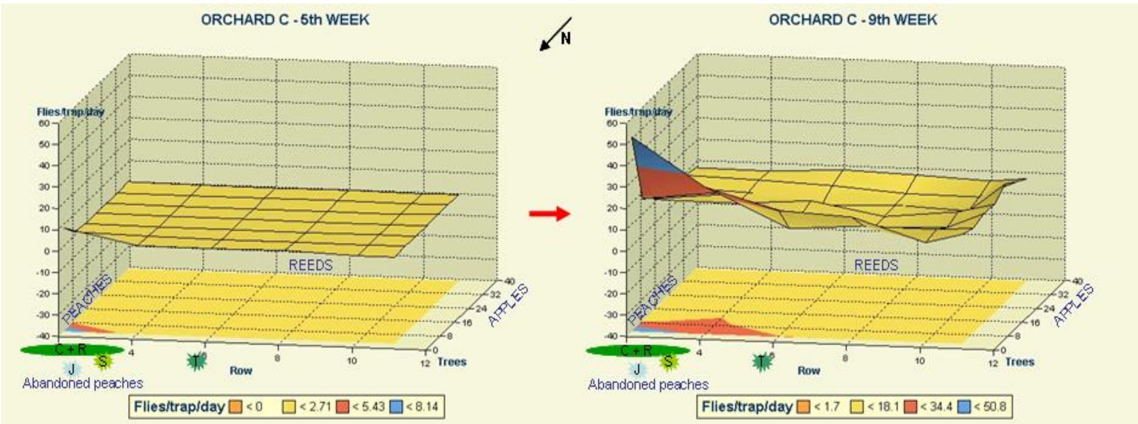


Figure 61. Spatial graphs of plot C showing the spatial distribution during the 5th and 9th weeks. The surrounding plots and vegetation are shown: C corresponds to cypress, R to blackberry, S to willow, T to salt cedar and J to walnut.

Analysis of the results for the four plots monitored, showed that field colonization by the pest usually started near to one or more plot edges, and from there, spread throughout the orchard. In three cases, capture levels at the edges were consistently higher than in the inner part of the orchard though in the remaining case, for several weeks, captures were higher in the inner area.

3.2 EXPERIMENT 2. LEVEL OF PROTECTION PROVIDED FOR THE FRUIT BY THE MASS TRAPPING TECHNIQUE AND INSECTICIDES

3.2.1 Population density captured in traps

Traps in orchards E1, E2, F and G were hung in the trees from 22 to 41 days but the total number of captures was extremely low (Table 25).

The percentage of females was higher than that of males. In the three cases where adults were sexed there was an average of 76.8% of females.

Table 25. Variety of peach trees, number of individuals captured in traps and percentage of females registered. In the checking of plot G (*) not all individuals were sexed.

PLOT	VARIETY	TOTAL CAPTURES	FEMALE PERCENTAGE
E1	'Elegant Lady'	76	57.89
E2	'Elegant Lady'	16	87.50
F	'Elegant Lady'	167	85.03
G	'Symphonie' & 'E. Lady'	8	*

3.2.2 Damage level

After reviewing 500 fruits/ha in each of the four plots, the damage level found in all of them was zero: no evidence of larvae was found in any of the fruits.

In plots where the pest control was reinforced by two or three chemical treatments (E1, E2 and G), captures were lower than those registered in the plot in which no treatment was realized (F), despite the presence of any damaged fruits.

3.3 EXPERIMENT 3. PROPORTION OF TRAPS TO BE CHECKED

The average number of captures of *C. capitata* adults during the nine weeks of the trials ranged between 0 and 24 F/T/D (Table 26). This maximum corresponded to 8,400 medflies/ha/week, assuming 50 traps/ha.

Taking into account all plots and all weeks, the error associated to each subset of traps ranged from 0 to 100%, when the size of the subset ranged from 90 to 20% of the total number of traps. As an example, the error values in the plot A1 for each week depending on the sample size of traps to be checked are shown (Table 27).

Table 26. Average number of flies/trap/day in the peach plots. The bold cells correspond to the various harvest times.

	PLOT (VARIETY)			
SAMPLING WEEK	**A1 ('Early O'Henry')**	**A2 ('Merryl O'Henry')**	**B ('Merryl O'Henry')**	**C ('Merryl O'Henry')**
2-8/07/07	0.19	0.16	0.06	0.11
9-15/07/07	0.10	0.07	0.07	0.16
16-22/07/07	0.18	0.13	0.08	0.11
23-29/07/07	0.41	0.14	0.25	0.23
30-5/08/07	0.92	0.40	0.56	0.54
6-12/08/07	**2.14**	0.55	1.10	1.11
13-19/08/07	**3.10**	0.39	**1.56**	1.09
20-26/08/07	**11.61**	**2.35**	**4.00**	**4.41**
27-2/09/07	**23.78**	**0.61**	**18.32**	**9.82**
Average	4.71	0.53	2.89	1.95

Taking into account all plots, at the high error (25%), the percentage of traps to be checked must be between 52 and 85%. At the low error point (10%), a higher percentage of traps need to be checked, between 68 and 92% (Table 28).

The period in which fruit from both fruit-species studied was sensitive to the pest attack lasted nine weeks. Taking into account the data from the whole plot throughout the research period, it was observed a difference between the proportion of traps to be checked over the whole period and those checked in the second half, when the fruit was ripe (Table 29). This differentiation in the production period was applied to all plots.

In any of the four plots studied was not possible to achieve a reduction in the percentage of peripheral traps to be checked during the entire period, analysing data obtained from traps at the periphery and in the inner area separately (Table 30).

Choosing data from the last five weeks, it was possible to achieve only a reduction of 10% in the peripheral traps to be checked (Table 30). Therefore, the idea of checking the peripheral and the inner traps separately was rejected.

Table 27. Sample size (percentage of traps selected, from the 98 traps hung) and its associated error for each checking date in the plot A1.

SAMPLE SIZE (%)	DATE								
	03/7/07	**10/7/07**	**16/7/07**	**23/7/07**	**30/7/07**	**06/8/07**	**14/8/07**	**20/8/07**	**28/8/07**
20	67.08	68.52	70.72	62.64	54.10	60.22	43.78	37.32	25.24
25	64.50	61.36	59.06	57.38	50.84	53.14	35.74	29.46	17.82
30	61.80	64.58	55.90	50.62	43.42	46.02	29.34	23.56	12.86
35	54.96	57.58	56.76	45.68	38.26	41.22	24.30	18.18	8.42
40	52.46	58.12	52.58	39.96	33.14	37.50	20.50	14.36	5.18
45	49.34	50.68	44.68	36.50	26.80	32.70	15.18	10.14	3.10
50	43.84	44.06	38.36	31.48	22.30	26.78	10.52	7.18	2.12
55	38.60	36.38	35.02	24.78	16.88	21.82	7.80	4.30	0.76
60	33.84	36.64	30.96	20.18	13.54	16.52	4.92	1.98	0.32
65	29.94	29.98	28.48	15.90	9.68	12.02	3.04	1.02	0.10
70	22.44	21.80	19.74	11.46	6.02	7.80	1.36	0.36	0.06
75	15.98	14.20	14.22	7.58	2.78	4.40	0.48	0.02	0.00
80	10.52	10.52	8.24	3.16	1.18	1.76	0.14	0.00	0.00
85	4.24	4.76	3.86	0.76	0.34	0.86	0.04	0.02	0.00
90	1.40	1.22	0.66	0.12	0.08	0.14	0.00	0.00	0.00

Table 28. Average percentage of traps to be checked taking in account the whole period, assuming error of 10 or 25%.

PLOT	VARIETY	SURFACE (ha)	ERROR (%)	
			10	**25**
A1	'Early O'Henry'	2.06	68.03	52.15
A2	'Merryl O'Henry'	1.93	72.00	57.30
B	'Merryl O'Henry'	0.85	90.00	84.90
C	'Merryl O'Henry'	0.72	92.10	85.20

Table 29. Average percentage of traps to be revised taking in account the whole period surveyed and the last part of it, and assuming the lowest percentage of error (10%).

PLOT	SAMPLING PERIOD	
	9 weeks	**5 last weeks**
A1	68.03	56.12
A2	72.00	62.40
B	90.00	87.20
C	92.10	89.50

Table 30. Percentage reduction of the peripheral traps to be checked taking into account the whole surveyed period (9 weeks) or the second half of it (last 5 weeks).

NUMBER OF WEEKS	% OF REDUCTION
9	None
5	10

The relationship between the average F/T/D and the percentage of traps to be checked was analyzed. Figure 62 shows the average number of F/T/D and the percentage of traps to be checked assuming two different errors in plots A1 and A2. These plots were situated in the same orchard, only eight meters apart and contained two varieties of peach with harvests differing by 14 days in 2007.

During the second period, the earlier variety achieved higher captures than the other and consequently, as it is shown in the graph, the number of traps to be checked during the latest five weeks was smaller.

In A1, during the early part of the period in question, optimum temperatures existed for the development of the pest, and adults could lay the eggs on the fruit because this plot was planted with the earliest variety. At the end of the period, although fruit had been harvested, the second generation autochthonous from the orchard was already at the adult stage, which probably explains the high captures during the final weeks.

Lines for the percentage of traps to be checked, assuming an error of 10 or 25% were parallel. By focusing on the lower risk (25%) the percentage of traps to be checked was smaller than for the higher risk (10%).

The other two plots were smaller than 1 ha, and both were cultivated with ‘Merryl O’Henry’ variety. In the corresponding graphs (Figure 63) there was one week when both lines of the percentage of traps to be checked assuming 10 or 25% of error were not parallel and crossed. This means that during that week it was assumed an error of 25%. That might have been due to different factors, i.e., the aggregate distribution of the pest, the low initial level or the reduced dimensions of the plots. In these cases, therefore, it was also showed the line corresponding to the percentage of traps to be checked assuming an error of 5%. In both cases this is the error that must be taken into account, although it only saved 10% of the traps to be checked.

Capture levels had similar tendencies in both orchards, except that, in the last monitoring, plot B registered almost double the number of individuals, compared with the orchard C (Figure 63).

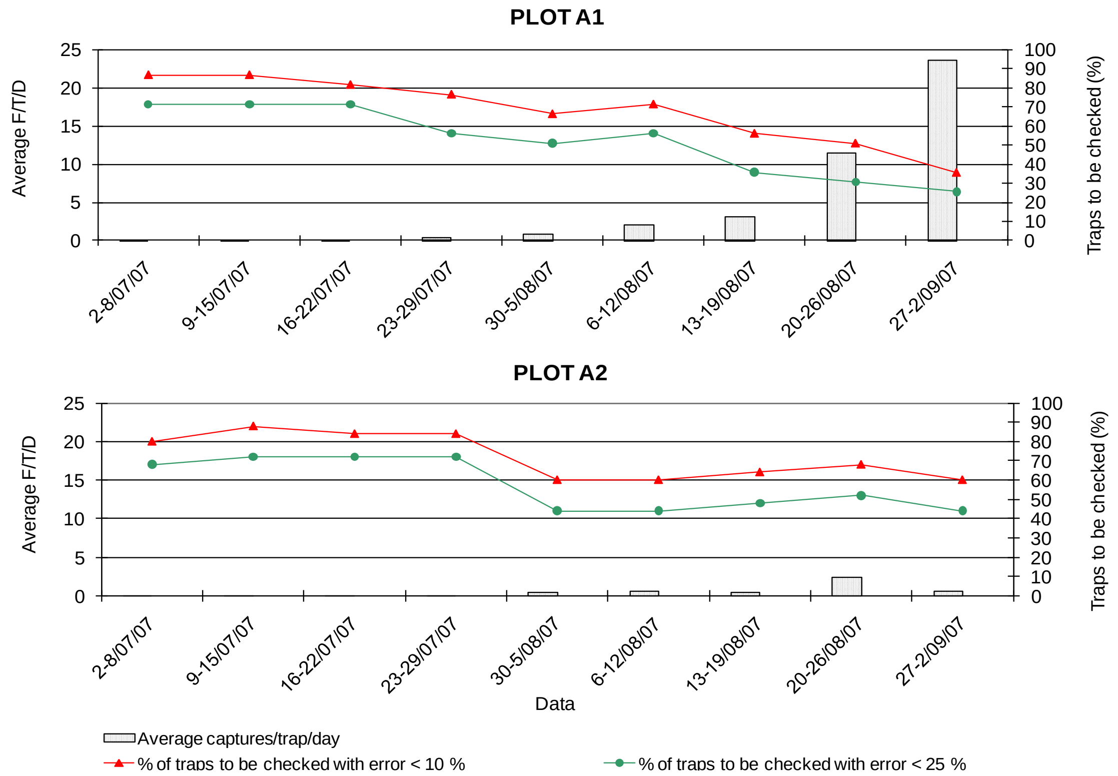


Figure 62. Average number of F/T/D and percentage of traps to be checked assuming two different errors in Plots A1 and A2.

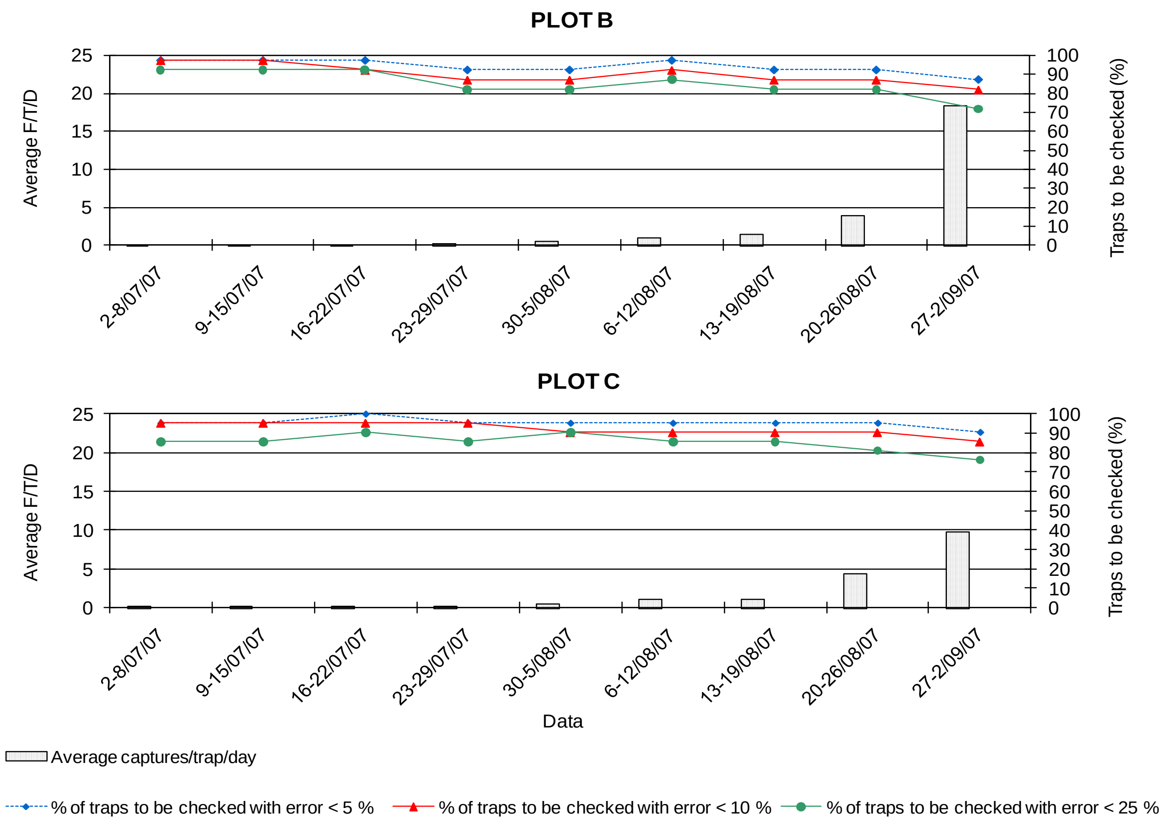


Figure 63. Average number of F/T/D and percentage of traps to be checked assuming two different errors in Plots B and C.

4 DISCUSSION

4.1 EXPERIMENT 1. COLONIZATION PROCESS AND SPATIAL DISTRIBUTION

The importance of studying the population dynamics of *C. capitata* has been recognised for more than twenty years and has been described in many studies around the world, including Chile (Harris and Olalquiaga, 1991), France (Cayol and Causse, 1993), Ghana (Appiah et al., 2009), Greece (Papadopoulos et al., 2003) (Nestel et al., 2004), Italy (Trematerra et al., 2008), Israel (Nestel et al., 2004) (Israely et al., 2005), Peru (Harris and Olalquiaga, 1991), Spain (Ros et al., 1999) (Alemany et al., 2006) (Martinez-Ferrer et al., 2006) (Escudero-Colomar et al., 2008) and United States (Hawaii) (Harris et al., 1993). The present work focused on the population dynamics at plot level in a fruit growing area from the North-East extreme of Spain.

Because of the importance of choosing the correct moment to place the traps in order to reduce the proportion of damage-producing medfly females (Cohen and Voet, 2002), it was elected to install them after the first capture of medfly adults in the area (23rd May, 2007). It was also taken into account that the releasing period of the attractant dispenser and insecticide is 120 days, which covered the whole ripening period without the need to replace them.

Analysis of adult captures/ha from the studied orchards makes entirely plausible the much higher values associated with the earlier peach variety (plot A1) specifically seven times more captures/ha than in the nearest orchard (plot A2) and twice as many as in the other two orchards (plots B and C). These differences confirm the hypothesis that the earlier fruit cultivars play an important role in subsequent population development (Katsoyannos et al., 1998). It is also reasonable to assume that catching as many females as possible during the ripening period of the early varieties provides positive protection for the later ripening cultivars.

Food baits, such as those used in this study, have high selectivity to females and can directly reduce the number of pre-reproductive females, which makes them a useful tool for fruit fly control (Lux et al., 2003). The average proportion of females captured in all studied plots was higher than the average for males, and the ratio males/females/trap/day taking into account the average of all weeks studied and all plots also confirmed the predominance of females, with the consequently positive effect. The fact that females were more often captured could also be due to the feeding events more commonly observed among females than males (Hendrichs and Hendrichs, 1990), resulting in a higher probability of their being captured.

Recent studies of citrus, peach and vine used the same trap (Maxitrap®) and attractant but administered in one dispenser, Ferag® CC D TM (SEDQ S.L.) rather than the three dispensers used in the present trial (Alonso-Muñoz and García-Marí, 2007) (Lucas and Hermosilla, 2008). Both studies found higher levels of captured females (70.47 to 83.05% of females) than those found in this experiment. Another assay performed on citrus groves used Maxitrap® baited with BioLure® Med Fly attractant (Suterra España Biocontrol S.L., Cerdanyola del Vallès, Spain) and the percentage of females found (68.81%) was at the same level as the highest registered in the present work (Navarro-Llopis et al., 2008). Other publications using different trap model (Tephri-trap®) baited with ammonium acetate, trimethylamine and putrescine (BioLure® or Tripack™, Kenogard S.A., Barcelona, Spain) also corroborated the higher level of captured females (Ros et al., 1997) (Epsky et al., 1999) (Miranda et al., 2001) (Martinez-Ferrer et al., 2006).

At the end of the study period, in three out of four cases, the ratio males to females was higher, and although the final weeks of the experiment took place in late summer, these results were comparable with other findings (Ros et al., 2002), which suggest that male populations would increase during autumn.

The higher proportion of females at the beginning of the season and the latest change in the dominant gender found in the experiment were also observed in a peach orchard cultivated with several varieties including O'Henry (Sastre et al., 1999). These authors studied the captures of medfly over eight weeks, from early July until the first week of September and they found that at the beginning of the period, captures were almost exclusively females and later, this percentage decreased until almost identical to the proportions of males (Sastre et al., 1999).

Comparable results for the different temporal patterns of male and female *C. capitata* were found in a mixed orchard in Northern Greece (Papadopoulos et al., 2003). From early September until the beginning of October, female captures were higher than those for males. However, from mid October until the end of November, male captures rose until they became dominant (Papadopoulos et al., 2003). In this last study, the different spatial pattern for males and females suggested that the sexes responded differently to the spatial and temporal environmental variability found in the orchard. Female spatial aggregation was closely related to the phenology of the host tree and to the sequential availability of ripe or semi-ripe fruits in the orchard. Males, however, in addition to foraging for food, probably concentrate on areas that provide appropriate shelters and sites to exhibit calling and lekking behaviour (Hendrichs et al., 1991) (Papadopoulos et al., 2003). Data analysis of all four plots of the present study agreed with the previous findings: while peach trees had plenty of ripening fruits, captures of females were more abundant inside the tree canopy.

Although it is difficult to detect infestation on peach fruits because of their colour (Cayol and Causse, 1993), some larval damage to fruits was detected. The damage level economically tolerable for fruit growers was the same as that accepted in other surveys which used different traps against Tephritid fly species on apple orchards (Prokopy et al., 1990). Damage levels registered in plots A1, A2 and B were greater than the above value (1%) despite the different control methods used. This could be due to the fact that during 2007 there was a high pest population in the Spanish Mediterranean fruit area and specifically in peach trees from Girona (Escudero-Colomar et al., 2008).

Because the population can increase suddenly, during a critical period (the last few weeks before harvesting) it would be useful to service the fruits with daily frequency (Romani, 1997), looking for the first fertile rotten punctures (with larvae inside the fruit).

During the first experiment one chemical treatment was carried out in one of the analyzed plots and two more in the other three plots. The treatments average of the four peach orchards in which mass trapping was used was 1.75. This is a very similar value to the 1.85 found during the same year in peach orchards managed with mass trapping in the Spanish fruit growing area of Lleida (Torà, 2008).

In the mass trapping study performed a decade ago in a peach orchard with the variety ‘O’Henry’ and using Tephri-trap® baited with ammonium acetate, trimethylamine and putrescine, the percentage of damage (0.50%) was lower than the one registered in the present study (Sastre et al., 1999). In 2006, six plots cultivated with the ‘Merryl O’Henry’ variety were evaluated in the same area as the present work and the damage level detected was also lower (varying from 0 to 0.69%) than the one obtained in this study (Batllori et al., 2008). Nevertheless, these differences between damage levels were consistent with the differences between population levels in Girona peach orchards, which during 2007 were twice as large as the previous year (Escudero-Colomar et al., 2008).

In order to reduce fruit damage it would be advisable to test the impact of an increase in the number of traps/ha.

When three-quarters of the fruit harvest had been completed in plot A1, captures in A2 were six times higher than for the previous week. This could be due to non migratory appetitive movements (searching for food) (Aluja, 1993), when part of the population moves towards available resources, from A1 to A2 and other closer orchards. This hypothesis is confirmed by the results of a study in which it was found that the average distance flown by *C. capitata* was extremely short and that 90% of adults displaced only 400 - 700 meters (Meats and Smallridge, 2007). Moreover, it is known that fruit flies can adjust their foraging behaviour according to the nutritional conditions prevailing in the orchard, such as presence or absence of ripe fruits in the area (Hendrichs et al., 1991) (Nestel et al., 2004) (Manrakhan and Lux, 2008) (Appiah et al., 2009).

In the last week of the studied period, when two-thirds of the harvest from A2 was finished, the average F/T/D in the latest variety fell by a multiple of seven when compared with the previous week (8^{th}). This could be due to the fact that flies were again searching for new resources.

Population movement between plots A1 and A2 could also be explained by a 3-year study in Hawaii, in which it was found that a small segment of the population always disperses into surrounding areas and when conditions are favourable they start populating the new area (Harris et al., 1993).

Comparable observations of variations between neighbouring plots were described in a study of two adjacent citrus orchards (500m in between) using Jackson traps (Katsoyannos et al., 1998). From June to August and during the three years studied the insect's population dynamics varied in the two orchards, perhaps due to differences in the host's composition, with nearly twice as large captures in the plot which contained early maturing hosts (Katsoyannos et al., 1998).

Another study between two heterogeneous orchards that differed in abundance and availability of host fruits and were separated by 500m, showed a population fluctuation remarkably different in each plot, and this difference remained consistent throughout the three years analysed (Papadopoulos et al., 2001). In the current experiment, there were 7.6 times as many captures in the plot with an early maturing host, as in its neighbouring plot, where harvesting took place a fortnight later. The damage level registered in this study was much greater in the first plot mentioned (Papadopoulos et al., 2001).

In 2007, the adult population in Girona fruit area appeared very early in the season. The first capture was on 23^{rd} May, one month earlier than the first one of the previous year. That led to the development of a complete generation in the earliest varieties of peach, because larvae require only 10 - 15 days to reach maturity in a green peach (Weems, 1981) and 17 days at 20ºC for the pupae development (Duyck and Quilici, 2002). The last week's captures were extremely elevated in the early variety, without commercial fruit, which could be due to the autochthonous population, reared on the fruits from 'Early O'Henry', not an immigrant one. Peaches are high protein fruits and it is known that larval diet with protein supplement facilitate the more rapid development of this species (Kaspi et al., 2002). This corroborates the possibility of a quick development of autochthonous individuals.

Other factors are involved with the hypothesis of an autochthonous population, such as the temperature pattern registered during the study period. Over the nine weeks evaluated, the average temperature varied between 16ºC and 25.2ºC, the lowest minimum temperature being 11.9ºC and the highest maximum temperature 31.6ºC. All these conditions were above the zero point of development for egg, larva and pupa stage (10ºC) (Weems, 1981), above the estimated thresholds: egg (9.9ºC), larva (5.2ºC) and pupa stage (9.1ºC) (Vargas et al., 1996) and above the lower developmental threshold for egg (11ºC), larva (5ºC) and pupa stage (13ºC) (Shoukry and Hafez, 1979). Studies of the biology of the medfly performed in order to determine these last thresholds were conducted in Egypt, with flies obtained from the laboratory colony maintained on an artificial carrot medium at 25ºC and 60% R.H. (Shoukry and Hafez, 1979). It would be necessary to do biological studies with wild flies from Girona fruit growing area, in order to verify whether thresholds are the same as in other regions or if populations adapt depending on the area.

Cultural measures are an important tool in the management of the target pest (Manrakhan and Lux, 2008), and poor orchard sanitation has been identified in other studies as a possible factor contributing to the increasing adult population (Appiah et al., 2008). Maintaining the traps during the period after the harvest helped prevented the spread of the new generation reared in the non commercial fruits that had not been immediately destroyed. When these adults emerged and there was no available fruit in either of the orchards (‘Merryl O’Henry’ had already been harvested) effective mass trapping was performed, as has been observed in other studies (Martínez-Ferrer et al., 2007). Traps competed favourably with the remaining fruits on the trees or in the soil and captured part of the population. This report and another realized in the same area (Batllori et al., 2008) confirmed the high importance of grinding the non commercial fruit prior to larval emergence (Prokopy et al., 1990) (Quilici et al., 2005). It was also confirmed the maintenance of traps after the harvest period to reduce within-orchard populations to a low level and to avoid a future infection focus for nearby plots.

In plot B, maximum captures were detected between the second and last weeks in the borders close to the irrigation channel and the fig tree. A recent study performed in Girona fruit area showed the importance of water courses in the capture of *C. capitata*. A correlation was found between population level and the distance between orchards and wet areas, this being stronger at shorter longitudes (Benejam, 2008). The attraction of the medfly to water courses is a possible explanation for the higher number of captures in the corner surrounded by irrigation channels.

It has been demonstrated by several authors that the fig tree is a heavily infested host (Katsoyannos et al., 1998) (Ros et al., 1999) (Papadopoulos et al., 2001) (Alemany et al., 2004) (Segura et al., 2004) (Martinez-Ferrer et al., 2006) (Campos et al., 2007). Adult flies have been observed foraging for food throughout most of the day on fig and non-host foliage as well as on the figs themselves (Hendrichs et al., 1991), perhaps searching for the nitrogen present in fig fluids (Hendrichs and Hendrichs, 1990). More than a decade ago, a study performed in Israel showed that it is reasonable to assume that there will be a continuous flow of flies from abandoned figs into commercial apple orchards (Israely et al., 1997). In a recently Spanish study of the influence of nearby fig trees on captures of the pest (Alonso-Muñoz et al., 2008), the presence of isolated fig trees in citrus orchards was shown to have repercussions in the captures of traps sited within a distance of 10 - 50 m. This increase in captures was observed for most of the year but particularly in September and October.

In the present survey it was able to corroborate this assumption because plot B had an isolated fig tree in one corner and the level of captures was higher in nearby traps. Therefore, fig trees could be a dissemination point for *C. capitata* on peach fruits in the Girona area with a ripening period coincident with the fig. Another spatial and temporal analysis (through kriging interpolation) performed in a citrus orchard in Mallorca revealed the impact of unmanaged auxiliary host crops (fig trees) on pest development (Alemany et al., 2006). As in the findings from this study, the largest captures were obtained near the fig trees situated in a border of the orchard and individuals spread progressively inwards until the entire plot had been invaded.

Before implementing a fruit fly management programme it is essential to determine the species composition of the area (Manrakhan and Lux, 2008). In sub-tropical and tropical Tephritid (belonging to the genus *Ceratitis*, *Anastrepha* and *Dacus*) whose hosts are less predictable or highly patchy in distribution and abundance in space and time, conventional mating encounter sites may shift, not only between different parts of the host plants but also between host and non-host plants (Prokopy, 1980). As a basis for this report, the surroundings of plot C were examined and some of the species identified were included in the host list of *C. capitata*: blackberry was cited as a rarely infested host, willow was classified with an infestation level of unknown importance and walnut was identified as an occasionally infested host (Liquido et al., 1991).

Although blackberry has been identified as an infrequently infested host and no larvae emerged from the fruits collected in this trial, in a study of thirty larval hosts of medfly, this species was described as the one with a higher percentage of survival (66%) in pre-adult stages (Krainacker et al., 1987).

The other species identified on the Northern edge are not hosts of medfly but both (cypress and salt cedar) are used as refuges for birds such as the cattle egret, *Bubulcus ibis* (Linnaeus), the common magpie, *Pica pica* (Linnaeus) and the european starling, *Sturnus vulgaris* Linnaeus. There is evidence that the odour of avian faeces, a principal natural proteinaceous food that medfly adults use as a source of ammonia (compound of nitrogen), attracts them to plants that are not permanent hosts (Prokopy et al., 1996). It is known that males prefer to perch on leaves near bird droppings, and that females are attracted to the faeces primarily as a source of nutrition and not as a rendezvous site for mating (Shelly and Kennelly, 2007). Several species from the Tephritidae family have been shown to be attracted to chemical volatiles released from avian faecal material, such as *Anastrepha suspensa* (Loew), *Dacus dorsalis* Hendel and *C. capitata* (Christenson and Foote, 1960) (Hendrichs and Hendrichs, 1990) (Hendrichs et al., 1991) (Prokopy et al., 1993) (Epsky et al., 1997) (Warburg and Yuval, 1997). Protein deprived flies are positively attracted to food sources containing natural protein, such as chicken faeces (Manrakhan and Lux, 2008).

Abandoned orchards with incomplete harvesting (fruits in state of advanced ripeness on the soil surface or hanging on the trees) provide adults of *C. capitata* with refugee from control efforts and serve as source of infestation of neighbouring commercial plots (Cohen and Yuval, 2000) (Appiah et al., 2008) (Trematerra et al., 2008). Therefore, the abandoned plot located behind the Northern side of plot C is also (like the other trees and shrubs) a possible infestation focus for the pest and this is confirmed by the fact that this border had the highest number of captures in the entire trial.

The border-effect observed in three out of four cases in this study has been found also in a trial conducted over eight weeks in a peach orchard in Tarragona planted with several varieties, one of which was O'Henry (Sastre et al., 1999). In this study the dosage of traps was higher than in the current one, and twice as high in the borders (125 traps/ha in total). Those authors reported that damaged fruits were always on the periphery of the plot (Sastre et al., 1999). Other mass trapping studies against *C. capitata* conducted in two citrus groves in Spain also demonstrated the significance of the border-effect (Alonso-Muñoz and García-Marí, 2004) (Alemany et al., 2006), registering lower captures in the traps located in the inner part of the plots. A strong border-effect was also observed in seven mass trapping trials carried out on apple fruit orchards in Girona Province from 2004 to 2006 (Escudero-Colomar et al., 2010).

The high variability of adult captures found in adjoining traps on all studied plots could be due to several factors already analyzed, including the proximity of a host tree or abandoned orchards, the border-effect, and other factors such as the predation suggested in a mass trapping trial on citrus trees (Campos et al., 2007), where an elevated ant level was observed.

4.2 EXPERIMENT 2. LEVEL OF PROTECTION PROVIDED FOR THE FRUIT BY THE MASS TRAPPING TECHNIQUE AND INSECTICIDES

In the three plots where adult captures where sexed, the percentage of females was higher than males, corroborating the findings from the first experiment, which was performed on peach orchards with later varieties than those analyzed in this trial.

Comparing the damage level registered in the pre-harvest fruit evaluation performed in the first experiment, opposite results were obtained in these four trials, where any fruit damage was found. This absence of damage was also found in an evaluation performed at the same area as the present work during 2006, on a plot also cultivated with the 'Elegant Lady' variety and under mass trapping management (Batllori et al., 2008).

In the current experiment one plot had no need for chemical reinforcement, two other orchards required two treatments, and the last received three, though none of them presented symptoms of the pest.

During the growing season of 2008 in Girona fruit growing area, the recorded population level of *C. capitata* was higher than in previous years (Escudero-Colomar et al., 2009). However, the study performed in 2.7 ha of this area produced extremely low level of captures, maybe due to the fact that the analyzed varieties corresponded to earlier ripening periods than those analyzed in the previous experiment, and during the surveyed months their maturity period did not coincide with the presence of an elevated population.

Therefore, the absence of damage to varieties ripened in the middle of the fruit season in all these plots might be due to several factors, including the efficiency of the mass trapping system, reinforcement with chemical treatments (when they were applied) and the low population density registered during the study period of 2008 in the survey plots.

The average number of captures of *C. capitata* adults during the experimental period (from 0 to 24 F/T/D) registered in the North-East of Spain was within the same order as the picks (maximum number of captures) registered in several Mediterranean citrus areas from 2003 to 2006. The maximum population level found in orchards from Valencia and the Balearic Island Ibiza corresponded to 25 F/T/D in mid July and end September, respectively (Martínez-Ferrer et al., 2007), while in the present study, pick values were double (from 50 to 58 F/T/D). The large differences between the pick values could be due to the generally elevated level of its populations in the Mediterranean area during 2007 and the irregular geographic distribution of the pest.

4.3 EXPERIMENT 3. PROPORTION OF TRAPS TO BE CHECKED

Analysis of the whole plot over the entire period identified a difference between the reductions available using different error values. At the high error level (25%) the percentage of traps to be checked must be between 52 and 85% of the total number of traps, while at the low error level (10%) the requirement ranged between 68 and 92%. However, as the aim in the pest control was to achieve a maximum threshold of 1% of damage caused by the species, it was important to choose the lower error value. The choice of the more conservative option was taken because of the heterogeneous spatial distribution found in this study and because the relation between the capture level and the real population present in the field is unknown.

If too small percentage of installed traps is checked, the error in the estimation of the population level will be large which could result in the recommendation of unnecessary chemical treatments, or failure to recommend them when they are essential.

Once the percentage of traps to be checked is known, it is important to determine their location. To identify the traps that must be checked, specific studies in the area are required. Due to the complexity of the pest’s distribution, it is necessary to conduct an accurate follow-up of the captures over the entire period, in order to discover the background of the captures in the plot (which are the orientations with higher percentage of captures, etc.).

5 CONCLUSIONS

Field colonization by the species *C. capitata* usually starts on the edge of the plot, and from there, spreads throughout the orchard. The percentage of females is higher than for males during the ripening period. Host plants, species that provide refuge for birds and water courses located at borders of the plot appear to be important factors in the spatial distribution of the pest and must always be considered when mass trapping is used.

Mass trapping technique is an effective method for the control of *C. capitata* in peach orchards in the North East of Spain when the population level is low but when it is normal or high it must be reinforced by chemical spraying.

The proportion of traps to be checked is inversely related to the population density. It is not possible to reduce differentially the number of traps on the periphery and the inside of the plot, and it cannot be reduced neither when the plot size is smaller than 1 ha. Nevertheless, when the plot size is larger than 1 ha it would be enough to check 60% of the traps during the last 5 weeks of the ripening period, or 70% over the full period.

6 REFERENCES

Alemany, A., Miranda, M.A., Alonso, R., Escorza, C.M., 2004. Efectividad del trampeo masivo de hembras de Ceratitis capitata (Diptera:Tephritidae) a base de atrayentes alimentarios. "Efecto-borde" y papel de los frutales abandonados como potenciadores de la plaga. Boletín de Sanidad Vegetal, Plagas 30, 255-264.

Alemany, A., Miranda, M.A., Alonso, R., Escorza, C.M., 2006. Changes in the spatial and temporal population density of the Mediterranean fruit fly (Diptera : Tephritidae) in a citrus orchard. Spanish Journal of Agricultural Research 4, 161-166.

Alonso-Muñoz, A., Arilla, J., Torres, J., Fernández, L., García, E., García-Marí, F., 2008. Influence of nearby fig trees on medfly captures of traps located in citrus orchards. First Meeting of TEAM, Palma de Mallorca 7-8 April, 2008, pp. 53.

Alonso-Muñoz, A., García-Marí, F., 2004. Control de Ceratitis capitata (Diptera: Tephritidae) en cítricos utilizando trampeo masivo. Phytoma España 157, 28-37.

Alonso-Muñoz, A., García-Marí, F., 2007. Comparación de cuatro tipos de mosqueros comerciales de captura de hembras y machos de Ceratitis capitata (Diptera: Tephritidae). Boletín de Sanidad Vegetal, Plagas 33, 399-407.

Aluja, M., 1993. The study of movement in Tephritid Flies: Review of concepts and recent advances. Fruit Flies: Biology and Management, pp. 105-113.

Appiah, E.F., Afreh-Nuamah, K., Obeng-Ofori, D., 2008. Relationship between fruiting phenology of Late Valencia citrus (Citrus sinensis) and abundance and distribution of Mediterranean fruit fly, Ceratitis capitata. International Society of Chemical Ecology, Pennsylvania, August 17-22, 2008, pp. 73.

Appiah, E.F., Afreh-Nuamah, K., Obeng-Ofori, D., 2009. Abundance and distribution of the Mediterranean fruit fly Ceratitis capitata (Diptera: Tephritidae), in Late Valencia citrus orchards in Ghana. International Journal of Tropical Insect Science 29, 11-16.

Batllori, J.L., Vilajeliu, M., Creixell, A., Carbo, M., Garcia, N., Esteba, G., Raset, F., Vayreda, F., Gine, M., Curos, D., Cornella, J., 2005. Reduction of insecticide sprayings by using alternative methods in commercial apple orchards. Bulletin OILB/SROP 28, 83-88.

Batllori, J.L., Vilajeliu, M., Vilardell, P., Creixell, A., Carbó, M., Esteba, G., Raset, F., Vayreda, F., Giné, M., Curós, D., 2003. Área piloto de reducción de insecticidas en plantaciones comerciales de manzano. Fruticultura Profesional 136, 49-54.

Batllori, L., Escudero, A., Vilajeliu, M., Garcia, F., Benejam, J., 2008. Area-wide mass trapping to control Ceratitis capitata (Wied.) on stone fruits in

Girona, NE of Spain. Integrated Plant Protection in Stone Fruit, IOBC/wprs Bulletin 37, 73-82.
Benejam, J., 2008. Resultats de dos anys de Lluita Obligatòria pel control de la mosca de la fruita (Ceratitis capitata) en presseguers de les comarques de l'Alt i el Baix Empordà. Universitat de Girona (non published document).
BOE, 2004. (Boletín Oficial del Estado) nº79 del 1 de abril del 2004, Real Decreto 461/2004, de 18 de marzo, por el que se establece el programa nacional de control dela mosca mediterránea de la fruta.
Broumas, T., Haniotakis, G., Liaropoulos, C., Tomazou, T., Ragoussis, N., 2002. The efficacy of an improved form of the mass-trapping method, for the control of the olive fruit fly, Bactrocera oleae (Gmelin) (Dipt., Tephritidae): pilot-scale feasibility studies. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 126, 217-223.
Cabrita, C., Ribeiro, J.R., 2006. Alternative methods for mass trapping of Medfly, C. capitata (Diptera: Tephritidae), in Algarve. Bulletin OILB/SROP 29, 99.
Campos, J.M., Fibla, J.M., Martínez-Ferrer, M.T., 2007. Efecto de frutales aislados en poblaciones de C. capitata en parcelas de cítricos. Levante Agrícola 385, 100-106.
Campos, J.M., Martínez-Ferrer, M.T., Fibla, J.M., 2008. Improvement of Ceratitis capitata mass-trapping strategies on citrus in north-eastern Spain. Control in Citrus Fruit Crops IOBC/wprs Bulletin 38, 144-149.
Carbó, J., Iglesias, I., Bonany, J., Casals, M., Dalmau, R., Pagès, J. Eds.), 2002. Melocotonero. Las variedades de más interés. Institut de Recerca i Tecnologia Agroalimentàries, Barcelona.
Carey, J.R., 1984. Host specific demographic studies of the Mediterranean fruit fly Ceratitis capitata. Ecological Entomology 9, 261-270.
Cayol, J.P., Causse, R., 1993. Mediterranean fruit fly Ceratitis capitata Wiedemann (Dipt., Trypetidae) back in Southern France. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 116.
Christenson, L.D., Foote, R.H., 1960. Biology of fruit flies. Annual Review of Entomology 5, 171-192.
Cohen, H., Voet, H., 2002. Effect of physiological state of young Ceratitis capitata females, on resource foraging behavior. Entomologia Experimentalis Et Applicata 104, 345-351.
Cohen, H., Yuval, B., 2000. Perimeter trapping strategy to reduce Mediterranean fruit fly (Diptera : Tephritidae) damage on different host species in Israel. Journal of Economic Entomology 93, 721-725.
DAR, 2008 Agrometeorologia. http://ruralcat.net.
DOGC, 2004. (Diari Oficial de la Generalitat de Catalunya) Nº4207. ARP/295/2004, en la que se establecieron medidas obligatorias de prevención y lucha contra la mosca mediterránea de la fruta (Ceratitis capitata Wiedemann).

Duyck, P.F., Quilici, S., 2002. Survival and development of different life stages of three Ceratitis spp. (Diptera : Tephritidae) reared at five constant temperatures. Bulletin of Entomological Research 92**,** 461-469.

Epsky, N.D., Dueben, B.D., Heath, R.R., Lauzon, C.R., Prokopy, R.J., 1997. Attraction of Anastrepha suspensa (Diptera: Tephritidae) to volatiles from avian fecal material. Florida Entomologist 80**,** 270-277.

Epsky, N.D., Hendrichs, J., Katsoyannos, B.I., Vasquez, L.A., Ros, J.P., Zumreoglu, A., Pereira, R., Bakri, A., Seewooruthun, S.I., Heath, R.R., 1999. Field evaluation of female-targeted trapping systems for Ceratitis capitata (Diptera : Tephritidae) in seven countries. Journal of Economic Entomology 92**,** 156-164.

Escudero, A., Vilajeliu, M., Batllori, L., 2005. Captura masiva para el control de la mosca mediterránea de la fruta (Ceratitis capitata Wied.) en manzano. Phytoma España 171**,** 26-31.

Escudero-Colomar, L.A., Vilajeliu, M., Batllori, L., 2008. Seasonality in the occurrence of the Mediterranean fruit fly [Ceratitis capitata (Wied.)] in the north-east of Spain. Journal of Applied Entomology 132**,** 714-721.

Escudero-Colomar, L.A., Vilajeliu, M., Batllori, L., 2009. Implicaciones de la dinámica poblacional de Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) en la aplicación de la captura masiva a gran escala. VI Congreso Nacional de Entomología Aplicada, Palma de Mallorca, 19-23 Octubre 2009.

Escudero-Colomar, L.A., Vilajeliu, M., Batllori, L., 2010. Mass trapping technique to control Ceratitis capitata (Diptera: Tephritidae) in apple orchards and study the distribution of the pest at field level. (under evaluation).

Fibla, J.M., Martínez-Ferrer, M.T., Campos, J.M., Monfort, R., Colell, R., 2007. Control de Ceratitis capitata Wied en variedades tempranas de cítricos y pequeñas superficies, mediante diferentes estrategias de trampeo masivo. Levante Agrícola 385**,** 126-134.

Harris, E.J., Olalquiaga, G., 1991. Ocurrence and distribution patterns of Mediterranean fruit fly (Diptera: Tephritidae) in desert areas in Chile and Peru. Environmental Entomology 20**,** 174-178.

Harris, E.J., Vargas, R.I., Gilmore, J.E., 1993. Seasonality in occurrence and distribution of Mediterranean fruit fly (Diptera: Tephritidae) in upland and lowland areas on Kauai, Hawaii. Environmental Entomology 22**,** 404-410.

Hendrichs, J., Hendrichs, M.A., 1990. Mediterranean fruit fly (Diptera: Tephritidae) in nature: location and diel pattern of feeding and other activities on fruiting and nonfruiting hosts and nonhosts. Annals of the Entomological Society of America 83**,** 632-641.

Hendrichs, J., Katsoyannos, B.I., Papaj, D.R., Prokopy, R.J., 1991. Sex differences in movement between natural feeding and mating sites and tradeoffs between food-consuption, mating success and predator evasion in Mediterranean fruit flies (Diptera: Teprhitidae). Oecologia 86, 223-231.

Israely, N., Yuval, B., Kitron, U., Nestel, D., 1997. Population fluctuations of adult Mediterranean fruit flies (Diptera : Tephritidae) in a Mediterranean heterogeneous agricultural region. Environmental Entomology 26, 1263-1269.

Israely, N., Ziv, Y., Oman, S.D., 2005. Spatiotemporal distribution patterns of Mediterranean fruit fly (Diptera : Tephritidae) in the central region of Israel. Annals of the Entomological Society of America 98, 77-84.

Kaspi, R., Mossinson, S., Drezner, T., Kamensky, B., Yuval, B., 2002. Effects of larval diet on development rates and reproductive maturation of male and female Mediterranean fruit flies. Physiological Entomology 27, 29-38.

Katsoyannos, B.I., Kouloussis, N.A., Carey, J.R., 1998. Seasonal and annual occurrence of Mediterranean fruit flies (Diptera : Tephritidae) on Chios Island, Greece: Differences between two neighboring citrus orchards. Annals of the Entomological Society of America 91, 43-51.

Krainacker, D.A., Carey, J.R., Vargas, R.I., 1987. Effect of larval host on life history traits of the Mediterranean fruit fly, Ceratitis capitata. Oecologia 73, 583-590.

Liquido, N.J., Shinoda, L.A., Cunningham, R.T. Eds.), 1991. Host plants of the Mediterranean fruit fly (Diptera: Tephritidae): an annotated world review. Entomological Society of America, Lanham, MD.

Lucas, A., Hermosilla, A., 2008. Eficacia de diferentes atrayentes y mosqueros para el control de mosca de la fruta (Ceratitis capitata) en uva de mesa. Agricola Vergel 27, 260-268.

Lux, S.A., Ekesi, S., Dimbi, S., Mohamed, S., Billah, M., 2003. Mango-infesting fruit flies in Africa: perspectives and limitations of biological approaches to their management. Biological Control in IPM Systems in Africa, 277-293.

Manrakhan, A., Lux, S.A., 2008. Effect of food deprivation on attractiveness of food sources, containing natural and artificial sugar and protein, to three African fruit flies: Ceratitis cosyra, Ceratitis fasciventris, and Ceratitis capitata. Entomologia Experimentalis Et Applicata 127, 133-143.

Martinez-Ferrer, M.T., Campos, J.M., Fibla, J.M., 2006. Population dynamics of Ceratitis capitata on citrus in northeast Spain: the influence of adjacent host fruit trees. Bulletin OILB/SROP 29, 77-84.

Martínez-Ferrer, M.T., Alonso, A., Campos, J.M., Fibla, J.M., Garcia-Marí, F., 2007. Dinámica poblacional de la mosca de la fruta Ceratitis Capitata en tres zonas citrícolas mediterráneas. Levante Agrícola 385, 1-7.

Meats, A., Smallridge, C.J., 2007. Short- and long-range dispersal of medfly, Ceratitis capitata (Dipt., Tephritidae), and its invasive potential. Journal of Applied Entomology 131, 518-523.

Miranda, M.A., Alonso, R., Alemany, A., 2001. Field evaluation of Medfly (Dipt., Tephritidae) female attractants in a Mediterranean agrosystem (Balearic

Islands, Spain). Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 125**,** 333-339.

Navarro-Llopis, V., Alfaro, F., Dominguez, J., Sanchis, J., Primo, J., 2008. Evaluation of traps and lures for mass trapping of Mediterranean fruit fly in citrus groves. Journal of Economic Entomology 101**,** 126-131.

Nestel, D., Katsoyannos, B., Nemny-Lavy, E., Mendel, Z., Papadopoulos, N., 2004. Spatial analysis of Medfly populations in heterogeneous landscapes. Proceedings of the 6th International Symposium on fruit flies of economic importance, Stellenbosch, South Africa, 6-10 May 2002**,** 35-43.

Papadopoulos, N.T., Katsoyannos, B.I., Carey, J.R., Kouloussis, N.A., 2001. Seasonal and annual occurrence of the Mediterranean fruit fly (Diptera : Tephritidae) in northern Greece. Annals of the Entomological Society of America 94**,** 41-50.

Papadopoulos, N.T., Katsoyannos, B.I., Nestel, D., 2003. Spatial autocorrelation analysis of a Ceratitis capitata (Diptera : Tephritidae) adult population in a mixed deciduous fruit orchard in northern Greece. Environmental Entomology 32**,** 319-326.

Prokopy, R.J., 1980. Mating behavior of frugivorous Tephritidae in nature. Proceedings of the Symposium on Fruit Fly Problems, XVI International Congress of Entomology, Kyoto, August 9-12, 1980, pp. 37-46.

Prokopy, R.J., Duan, J.J., Vargas, R.I., 1996. Potential for host range expansion in Ceratitis capitata flies: Impact of proximity of adult food to egg-laying sites. Ecological Entomology 21**,** 295-299.

Prokopy, R.J., Hsu, C.L., Vargas, R.I., 1993. Effect of source and condition of animal excrement on attractiveness to adults of Ceratitis capitata (Diptera: Tephritidae). Environmental Entomology 22**,** 453-458.

Prokopy, R.J., Johnson, S.A., Obrien, M.T., 1990. Second-stage integrated management of apple arthropod pests. Entomologia Experimentalis Et Applicata 54**,** 9-19.

Quilici, S., Duyck, P.F., Rousse, P., Gourdon, F., Simiand, C., Franck, A., 2005. La mouche de la pêche sur mangue, goyave, etc. Phytoma, la défense des végétaux. 584**,** 44-47.

Romani, M., 1997. Biology, ethology and control of Ceratitis capitata in northern Italy. Informatore Agrario 53**,** 59-62.

Ros, J.P., Castillo, E., Crespo, J., Latorre, Y., Martin, P., Miranda, M.A., Moner, P., Sastre, C., 1997. Evaluación en campo de varios atrayentes sintéticos para la captura de hembras de la mosca mediterránea de la fruta Ceratitis capitata Wied. (Díptera: Tephritidae). Boletín de Sanidad Vegetal, Plagas 23**,** 393-402.

Ros, J.P., Guirado, I., Escobar, I., 1999. Estudio poblacional de la mosca mediterránea de la Fruta (Ceratitis capitata Wied.) en los cultivos subtropicales de la costa de Granada. Boletin de Sanidad Vegetal, Plagas 25**,** 505-514.

Ros, J.P., Wong, E., Olivero, J., Castillo, E., 2002. Mejora de los mosqueros, atrayentes y sistemas de retención contra la mosca mediterránea de la fruta Ceratitis capitata Wied. Cómo hacer de la Técnica del Trampeo Masivo una buena herramienta para controlar esta plaga. Boletín de Sanidad Vegetal, Plagas 28**,** 591-597.

Sastre, C., Melo, J.C., Borreli, G., 1999. La captura de hembras: una posible salida en el control de mosca de la fruta (Ceratitis capitata, Wied.) en melocotonero. Phytoma España 113**,** 42-47.

Sciarretta, A., Cesare, D., De Salvador, R., Tabilio, M.R., Trematerra, P., 2009. Spatio-temporal distribution of Ceratitis capitata trap catches in an agricultural landscape. Pheromones and other Semiochemicals IOBC/wprs Bulletin 41**,** 123-129.

Segura, D.F., Vera, M.T., Cladera, J.L., 2004. Seasonal fluctuation on infestation of several hosts by the Mediterranean fruit fly, Ceratitis capitata (Diptera: Tephritidae) in Buenos Aires Province. Ecologia Austral 14**,** 3-17.

Shelly, T.E., Kennelly, S.S., 2007. Settlement patterns of mediterranean fruit flies in the tree canopy: An experimental analysis. Journal of Insect Behavior 20**,** 453-472.

Shoukry, A., Hafez, M., 1979. Studies on the biology of the Mediterranean fruit fly Ceratitis capitata. Entomologia Experimentalis Et Applicata 26**,** 33-39.

Torà, R., 2008. Mètodes biotècnics: la confusió sexual i la captura massiva. Perspectives de futur. Jornada Tècnica: Problemàtica actual en el control dels paràsits dels fruiters. Perspectives de futur, Lleida 26 Setembre, 2008, pp. 46.

Trematerra, P., Salvador, R.d., Cesare, D., Sciarretta, A., 2008. Spatio-temporal distribution of Ceratitis capitata trap catches and integrated pest management in an agricultural landscape of central Italy. Giornate Fitopatologiche 2008, Cervia (RA), 12-14 marzo 2008, Volume 1**,** 167-174.

Vargas, R.I., Walsh, W.A., Jang, E.B., Armstrong, J.W., Kanehisa, D.T., 1996. Survival and development of immature stages of four Hawaiian fruit flies (Diptera: Tephritidae) reared at five constant temperatures. Annals of the Entomological Society of America 89**,** 64-69.

Warburg, M.S., Yuval, B., 1997. Circadian patterns of feeding and reproductive activities of Mediterranean fruit flies (Diptera: Tephritidae) on various hosts in Israel. Annals of the Entomological Society of America 90**,** 487-495.

Weems, H.V., Jr., 1981. Mediterranean fruit fly, Ceratitis capitata (Wiedemann) (Diptera: Tephritidae). Entomology Circular, Division of Plant Industry, Florida Department of Agriculture and Consumer Services**,** 12 pp.

7. GENERAL DISCUSSION

INDEX

1 DISCUSSION OF RESULTS FROM A HOLISTIC PERSPECTIVE......... 197

2 REFERENCES 201

1 DISCUSSION OF RESULTS FROM A HOLISTIC PERSPECTIVE

One of the major pests of commercial fruits throughout the world, *Ceratitis capitata* (Wiedemann), induces the highest losses in the harvest of fruit crops in the Mediterranean area (Enkerlin and Mumford, 1997) (Malacrida et al., 2007).

It is therefore, essential to improve the efficiency of the programs included in integrated pest management, especially the technology (Jones et al., 2009). The recent banning at European level of some active ingredients used for the control of fruit flies (EEC, 1991), has promoted the use of more environmental friendly techniques which avoid deposits of harmful residues and also avoid resistance to chemical products. One of the methodologies included in IPM is the mass trapping system, which uses a food lure and an insecticide placed inside a trap, as an alternative to intensive chemical control in the fight against *C. capitata* (Sastre, 1999).

There are few references to the study of mass trapping technique in peach crops (Sastre et al., 1999) (Batllori et al., 2008) and the last chapter of the present document aimed to optimize the application of the methodology on peaches.

Captures of medfly adults in each month of the study period provide information on the phenology of flight and therefore, of their abundance over time. Careful checking of all traps placed in the survey plots provided consistent and useful data on the pest in the study area. In the present study it was found that invasions of medfly usually came from nearby orchards and they spread from the edge of the plots. It was also demonstrated that the mass trapping technique using Maxitrap® trap, the attractant Ferag® CC 3D TM and the insecticide Ferag® ID TM (DDVP), is effective in the control of medfly in peach orchards in the North East of Spain when the population level is low, but when it is high, it must be reinforced by chemical spraying. This study is the first to use the methodology described above for the evaluation of the mass trapping technique in peach orchards.

The proportion of traps to be checked to ensure a reliable estimate of the medfly population captured was inversely related to the population density found. It is necessary to check a high percentage of traps in both cases, in the last part of the ripening phase and over the full period.

Some factors are involved in the effectiveness of the mass trapping, including the size and shape of the plot, the plant species and variety planted, the presence of ripening fruit in the orchard, the period of the year, the length of time the traps are in the plot and the population abundance of medfly in the area over the time in question (Alonso-Muñoz and García-Marí, 2009b). Spatial and temporal distribution of captures are useful tools to understand the behaviour of the species and how well mass trapping is performing, although capture levels can be very variable between checks and between traps that are situated close to each other. Several factors were therefore considered in the current study, including host plants, species which provide refuge for birds and water courses located at borders of the plot. The results showed that these factors are important in the spatial distribution of the pest and must always be considered when mass trapping is used.

For the success of pest control, it is very important to combine the available techniques, and to avoid the use of a single methodology. Mass trapping system would therefore be backed up by cultural methods, including the destruction and elimination of non commercial fruits in the plots to prevent females from laying eggs on them (Batllori et al., 2008), a practice endorsed in another recent study (Alonso-Muñoz and García-Marí, 2009a).

Mass trapping technique involves the installation of a high density of traps in the crop to be protected and achieves a measure of protection by removing a high proportion of individuals from the medfly population (Howse et al., 1998). The equipment currently used in mass trapping has been improved over time to be used in monitoring (Heath et al., 1995) (Epsky et al., 1999), but this does not mean that it has been obtained the best possible trapping equipment.

An insecticide must be incorporated into the trapping equipment used for this purpose because otherwise, the effectiveness of the system drops significantly (Escudero-Colomar et al., 2008b). The current European directive 91/414 EEC on pesticide marketing (EEC, 1991) has made it necessary to find a replacement for DDVP, the chemical product which has been used over the last decades.

The importance of the presentation mode and position of the insecticide were demonstrated in this study. In the position trial, impregnation by movement in the base of the trap made the insecticide slightly more effective, and in the first trial of formulations, it was shown that disposition by movement impregnation in the lid was much more effective than when used in the dispenser. Further studies could confirm the best position for the impregnation of the insecticide, taking into account other factors, including the trap model.

The highly efficient killing action of the plastic prototype containing the formulation of deltamethrin and its good performance against low and high population levels of medfly are promising for the future of the mass trapping. What is more, the lack of damaged apples registered in the last trial confirmed the efficiency of the mass trapping using this formulation of deltamethrin. Further studies will need to be carried out, to test its effectiveness with other crops and varieties.

C. capitata is considered an important pests of deciduous fruit industry in Spain (Escudero-Colomar et al., 2008a) and jointly with the congener *Ceratitis rosa* Karsch, both are major pests in regions such as South Africa (Nyamukondiwa and Terblanche, 2010) and La Réunion Island (Duyck et al., 2008). In La Réunion, *C. rosa* is the main species which has displaced *C. capitata* (Duyck et al., 2004). The absence of treatments against *C. rosa* in peach and other susceptible hosts can result in high levels of damage, including complete loss of production (Quilici and Franck, 1999).

Field trials can only be carried out once a year. In order to compare the results obtained in the Girona area with a completely different region and crop, similar trials were performed in La Réunion, giving the possibility of carrying out these studies twice in a single year.

In the four comparative trials carried out in La Réunion, the most captured species was *C. rosa*, followed by *B. cucurbitae* and *C. capitata*. *C. rosa* has potential as an invader and it may become a cosmopolitan pest in the future (Baliraine et al., 2004). It is necessary therefore, to test and install new methodologies for the control of these Tephritid pests where they are already present.

Similar results of those found in these comparative trials were obtained in Spain in some trials carried out in citrus groves (Lucas et al., 2006) (Alonso-Muñoz and García-Marí, 2007) (Lucas and Hermosilla, 2008b), but contradictory results were reported in other studies also carried out in citrus in a similar area (Navarro-Llopis et al., 2008). Therefore, although in the present study BioLure® Med Fly obtained distinctly the best results, it is very important for the establishment and success of mass trapping in an area, to test the equipment in advance. Regarding the comparative trials of commercial complete equipments (systems), the results supported previous comparative studies performed in citrus groves (Miranda et al., 2001) (Lucas and Hermosilla, 2008a). Therefore, the systems consisting of BioLure® Unipak+Tephri-trap®+DDVP and Ferag® CC D TM+Maxitrap®+DDVP could be suitable for use in mass trapping in the studied region. However, this equipment must be studied with the currently available insecticide, deltamethrin instead of the banned DDVP.

There is very little written about the comparative effectiveness of insecticides used in mass trapping. However, the positive results recorded in the present study using deltamethrin as a substitute of DDVP was first studied in the comparative trial carried out in a mango orchard (Ros et al., 2005) and earlier trials conducted in the Girona area over the last few years. The findings of this research are very important because there is an urgent need to find a suitable substitute for the recently banned DDVP and this study points the way.

In the North-East extreme of Spain, medfly is in the border of its distribution area (Vera et al., 2002), and an early detection of the population is essential, in order to efficiently stop its development. For this purpose, knowledge of the specific conditions of overwintering is an important requirement as it is for the implementation of integrated pest management and its alternatives (Nyamukondiwa and Terblanche, 2010). Several studies have been conducted in other temperate areas of the Mediterranean basin (Papadopoulos et al., 1996) (Sciarretta et al., 2009), but there are differences of climate between these regions and the Girona area, being necessary to carry out studies in this one.

In the study of the survival of wild medfly larvae, the rate of infestation depended on the cultivar of the host fruit. The 'Golden Delicious' variety was much more susceptible than 'Granny Smith', as confirmed in previous studies (Romani, 1997) (Papadopoulos et al., 2001). This information would be very useful for growers who have orchards located in areas with high medfly population levels, and wish to know which is the most suitable variety to plant, although 'Granny Smith' is also used in the pollinating process.

Trials examining the survival of wild larvae and the overwintering of pupa demonstrated that minimum temperatures registered over the study periods were related to longer intervals between fruit sampling and larval exit, longer development times of larva and pupa stages and high mortality rates, as already observed by other authors (Shoukry and Hafez, 1979) (Aluja, 1993) (Papadopoulos et al., 1996) (Vargas et al., 1996) (Mavrikakis et al., 2000) (Duyck and Quilici, 2002) (Segura et al., 2004).

It is very important to confirm that mature larva jumped from 'Golden Delicious' fruits up to the third week of December and that the last emergence of adults from these pupae was in mid January. This made clear that the collection and destruction of fruits laying on the floor or left in the trees over winter, contribute to the diminution of the pest over this period, a critical factor in controlling the medfly population. However, in this study no adult emerged after mid January, and it was therefore impossible to prove that adults found in the following seasons were coming from fruits infested in late autumn or early winter. Further studies need to be carried out in the Girona region in order to verify the development stage and the place in which medfly survives through winter.

Some structural conditions of the soil and climatic factors appeared to be associated to the survival of pupae under winter conditions, including soils with low water holding capacity (Eskafi and Fernández, 1990), subsoil temperature (Feron and Guennelon, 1958), cold hours below a determined temperature (Denlinger and Lee, 1998) and the incidence of rainfall and temporary immersion (Duyck et al., 2006). Other factors did not seem to affect survival of medfly pupae, i.e. atmospheric humidity (Duyck et al., 2006).

Recent studies have shown that in the wild, *C. capitata* has the capability to adjust its thermal tolerance within a single generation over weekly and hourly time scales (Nyamukondiwa and Terblanche, 2010). However, in the study of the overwintering of adults, no individual survived the changing weather conditions registered during two winter seasons in Girona. Climatic conditions including low temperatures and high levels of rainfall were involved in the mortality of adults during winter.

2 REFERENCES

Alonso-Muñoz, A., García-Marí, F., 2009a. Daños causados por Ceratitis capitata (Diptera: Tephritidae) en parcelas de cítricos: comparación del ataque a frutos del árbol y a frutos en el suelo. Boletín de Sanidad Vegetal, Plagas 35**,** 543-557.

Alonso-Muñoz, A., García-Marí, F., 2009b. Factores que influyen en la eficacia del trampeo masivo para el control de la mosca de la fruta Ceratitis capitata (Diptera: Tephritidae). Boletin de Sanidad Vegetal, Plagas 35**,** 401-418.

Alonso-Muñoz, A., García-Marí, F., 2007. Comparación de cuatro tipos de mosqueros comerciales de captura de hembras y machos de Ceratitis capitata (Diptera: Tephritidae). Boletín de Sanidad Vegetal, Plagas 33**,** 399-407.

Aluja, M., 1993. The study of movement in Tephritid Flies: Review of concepts and recent advances. Fruit Flies: Biology and Management, pp. 105-113.

Baliraine, F.N., Bonizzoni, M., Guglielmino, C.R., Osir, E.O., Lux, S.A., Mulaa, F.J., Gomulski, L.M., Zheng, L., Quilici, S., Gasperi, G., Malacrida, A.R., 2004. Population genetics of the potentially invasive African fruit fly species, Ceratitis rosa and Ceratitis fasciventris (Diptera : Tephritidae). Molecular Ecology 13**,** 683-695.

Batllori, L., Escudero, A., Vilajeliu, M., Garcia, F., Benejam, J., 2008. Area-wide mass trapping to control Ceratitis capitata (Wied.) on stone fruits in Girona, NE of Spain. Integrated Plant Protection in Stone Fruit, IOBC/wprs Bulletin 37**,** 73-82.

Denlinger, D.L., Lee, R.E., 1998. Physiology of cold sensitivity. Temperature sensitivity in insects and application in Integrated Pest Management., pp. 55-95.

Duyck, P.F., David, P., Pavoine, S., Quilici, S., 2008. Can host-range allow niche differentiation of invasive polyphagous fruit flies (Diptera: Tephritidae) in La Reunion? Ecological Entomology 33**,** 439-452.

Duyck, P.F., David, P., Quilici, S., 2004. A review of relationships between interspecific competition and invasions in fruit flies (Diptera : Tephritidae). Ecological Entomology 29**,** 511-520.

Duyck, P.F., David, P., Quilici, S., 2006. Climatic niche partitioning following successive invasions by fruit flies in La Reunion. Journal of Animal Ecology 75**,** 518-526.

Duyck, P.F., Quilici, S., 2002. Survival and development of different life stages of three Ceratitis spp. (Diptera : Tephritidae) reared at five constant temperatures. Bulletin of Entomological Research 92**,** 461-469.

EEC, 1991. Council Directive of 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC).
Enkerlin, W., Mumford, J., 1997. Economic evaluation of three alternative methods for control of the Mediterranean fruit fly (Diptera: Tephritidae) in Israel, Palestinian territories, and Jordan. Journal of Economic Entomology 90**,** 1066-1072.
Epsky, N.D., Hendrichs, J., Katsoyannos, B.I., Vasquez, L.A., Ros, J.P., Zumreoglu, A., Pereira, R., Bakri, A., Seewooruthun, S.I., Heath, R.R., 1999. Field evaluation of female-targeted trapping systems for Ceratitis capitata (Diptera : Tephritidae) in seven countries. Journal of Economic Entomology 92**,** 156-164.
Escudero-Colomar, L.A., Vilajeliu, M., Batllori, L., 2008a. Seasonality in the occurrence of the Mediterranean fruit fly [Ceratitis capitata (Wied.)] in the north-east of Spain. Journal of Applied Entomology 132**,** 714-721.
Escudero-Colomar, L.A., Vilajeliu, M., Peñarrubia-María, E., Batllori, L., 2008b. Mass trapping as a control method for Medfly, in the NE of Spain, and improvements to the dry food based attractants. Consultant's Meeting on "Development of Bait Stations for Fruit Fly Suppression in Support of SIT", Mazatlán, Mexico.
Eskafi, F.M., Fernández, A., 1990. Larval-pupal mortality of Mediterranean Fruit Fly from interaction of soil, moisture, and temperature. Environmental Entomology 19**,** 1666-1670.
Feron, M., Guennelon, G., 1958. La Mouche des fruits dans la Vallée du Rhône. Jornées fruitières et Maraîchères d'Avignon. Avignon, France 24-25 January 1958. pp. 6.
Heath, R.R., Epsky, N.D., Guzman, A., Dueben, B.D., Manukian, A., Meyer, W.L., 1995. Development of a dry plastic insect trap with food-based synthetic attractant for the Mediterranean and Mexican Fruit-Flies (Diptera: Tephritidae). Journal of Economic Entomology 88**,** 1307-1315.
Howse, P., Stevens, I., Jones, O., 1998. Feromonas de insectos y su uso en el control de plagas.
Jones, V.P., Unruh, T.R., Horton, D.R., Mills, N.J., Brunner, J.F., Beers, E.H., Shearer, P.W., 2009. Tree fruit IPM programs in the western United States: the challenge of enhancing biological control through intensive management. Pest Management Science 65**,** 1305-1310.
Lucas, A., Fuentes, F., Hermosilla, A., 2006. Evaluación de la eficiencia de captura de adultos de mosca de la fruta (Ceratitis capitata) de diversos mosqueros y cebos, con y sin insecticida. Agrícola Vergel 294**,** 298-302.
Lucas, A., Hermosilla, A., 2008a. Eficacia de Ceratrap y otros atrayentes y mosqueros, en el control de mosca de la fruta (Ceratitis capitata) en cítricos. Agrícola Vergel 390**,** 159-166.
Lucas, A., Hermosilla, A., 2008b. Evaluación de la eficiencia en la captura de mosca de la fruta (Ceratitis capitata) de varios mosqueros y cebos, en cultivo de cítricos. Levante Agrícola 390**,** 169-178.
Malacrida, A.R., Gomulski, L.M., Bonizzoni, M., Bertin, S., Gasperi, G., Gugliclmino, C.R., 2007. Globalization and fruitfly invasion and expansion: the medfly paradigm. Genetica 131**,** 1-9.
Mavrikakis, P.G., Economopoulos, A.P., Carey, J.R., 2000. Continuous winter reproduction and growth of the Mediterranean fruit fly (Diptera :

Tephritidae) in Heraklion, Crete, southern Greece. Environmental Entomology 29, 1180-1187.
Miranda, M.A., Alonso, R., Alemany, A., 2001. Field evaluation of Medfly (Dipt., Tephritidae) female attractants in a Mediterranean agrosystem (Balearic Islands, Spain). Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 125, 333-339.
Navarro-Llopis, V., Alfaro, F., Dominguez, J., Sanchis, J., Primo, J., 2008. Evaluation of traps and lures for mass trapping of Mediterranean fruit fly in citrus groves. Journal of Economic Entomology 101, 126-131.
Nyamukondiwa, C., Terblanche, J.S., 2010. Within-generation variation of critical thermal limits in adult Mediterranean and Natal fruit flies Ceratitis capitata and Ceratitis rosa: thermal history affects short-term responses to temperature. Physiological Entomology 35, 255-264.
Papadopoulos, N.T., Carey, J.R., Katsoyannos, B.I., Kouloussis, N.A., 1996. Overwintering of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. Annals of the Entomological Society of America 89, 526-534.
Papadopoulos, N.T., Katsoyannos, B.I., Carey, J.R., Kouloussis, N.A., 2001a. Seasonal and annual occurrence of the Mediterranean fruit fly (Diptera : Tephritidae) in northern Greece. Annals of the Entomological Society of America 94, 41-50.
Quilici, S., Franck, A., 1999b. The Natal fruit fly: Ceratitis (Pterandrus) rosa Karsch. Diptera : Tephritidae. French name : la mouche du Natal. The Natal fruit fly: Ceratitis (Pterandrus) rosa Karsch. Diptera : Tephritidae. French name : la mouche du Natal, 2 pp.
Romani, M., 1997. Biology, ethology and control of Ceratitis capitata in northern Italy. Informatore Agrario 53, 59-62.
Ros, J.P., Wong, E., Olivero, J., Rubio, J.R., Marquez, A.L., Castillo, E., Blas, P., 2005b. Desarrollo de atrayentes y mosqueros para su integración en los programas de trampeo masivo contra la mosca de la fruta (Ceratitis capitata Wied.) y la del olivo (Bactrocera oleae Gmel.). Boletín de Sanidad Vegetal, Plagas 31, 599-609.
Sastre, C., 1999. Eficacia de los productos fitosanitarios en el control de la mosca de la fruta Ceratitis capitata (Wied.). Phytoma España 114, 75-77.
Sastre, C., Melo, J.C., Borreli, G., 1999b. La captura de hembras: una posible salida en el control de mosca de la fruta (Ceratitis capitata, Wied.) en melocotonero. Phytoma España, 42-47.
Sciarretta, A., Cesare, D., De Salvador, R., Tabilio, M.R., Trematerra, P., 2009. Spatio-temporal distribution of Ceratitis capitata trap catches in an agricultural landscape. Pheromones and other Semiochemicals IOBC/wprs Bulletin 41, 123-129.
Segura, D.F., Vera, M.T., Cladera, J.L., 2004. Fluctuación estacional en la infestación de diversos hospedadores por la mosca del Mediterráneo, Ceratitis capitata (Diptera: Tephritidae), en la provincia de Buenos Aires. Ecologia Austral 14, 3-17.
Shoukry, A., Hafez, M., 1979. Studies on the biology of the Mediterranean fruit fly Ceratitis capitata. Entomologia Experimentalis Et Applicata 26, 33-39.
Vargas, R.I., Walsh, W.A., Jang, E.B., Armstrong, J.W., Kanehisa, D.T., 1996. Survival and development of immature stages of four Hawaiian fruit flies

(Diptera: Tephritidae) reared at five constant temperatures. Annals of the Entomological Society of America 89**,** 64-69.

Vera, M.T., Rodriguez, R., Segura, D.F., Cladera, J.L., Sutherst, R.W., 2002. Potential geographical distribution of the Mediterranean fruit fly, Ceratitis capitata (Diptera : Tephritidae), with emphasis on Argentina and Australia. Environmental Entomology 31**,** 1009-1022.

8. GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

1. Stages of larvae and pupae of medfly collected from infested apples survived natural weather conditions of late autumn and early winter in the Girona fruit growing area but not throughout this period. Larval and pupa stages maintained in winter developed more slowly in comparison with individuals reared in a controlled environment. Adults continued to emerge until mid January.

2. No adult medfly emerged from pupae exposed throughout winter under natural conditions in either of the two years analyzed.

3. Medfly adults were unable to survive to the end of winter in the Girona fruit growing area in either of the studied years. Climatic conditions such as high levels of rainfall, low temperatures and strong winds appeared to be involved in the mortality of adults during winter.

4. The most captured species in all four trials carried out in La Réunion Island were *C. rosa* followed by *B. cucurbitae* and by *C. capitata*. Other fruit fly species were recorded in the trials and in order of relevance were *N. cyanescens*, *D. ciliatus*, *B. zonata*, *C. catoirii* and *D. demmerezi*.

5. The most effective traps for the capture of *C. rosa* and *C. capitata* were Maxitrap® and Tephri-trap® traps.

6. The most effective attractants for the capture of *C. rosa* were the dry food baits BioLure® Med Fly and BioLure® Unipak. Ferag® CC D TM, BioLure® Med Fly and BioLure® Unipak obtained the same results for the capture of *C. capitata*.

7. The formulation of insecticide deltamethrin at 15 mg dose tested in La Réunion could be a suitable substitute for DDVP, recently banned in the EU.

8. Systems composed of BioLure® Unipak+Tephri-trap®+DDVP and Ferag® CC D TM+Maxitrap®+DDVP performed effectively.

9. The formulation of insecticide deltamethrin at 20 mg dose tested in Girona could be a suitable substitute for DDVP.

10. Insecticide impregnated by movement in the base of the trap was slightly more effective than those placed in the lid. Further studies in respect of the position of the insecticide need to be conducted to confirm this finding.

11. The plastic prototype with the insecticide deltamethrin 12 mg had a highly efficient killing action at both low and high population levels in

mass trapping for the control of medfly. This formulation destroyed 98% of all flies entering the traps and is a suitable substitute for DDVP.

12. Field colonization by medfly usually starts at the edge of the plot and from there it spreads throughout the orchard. In peach orchards, the percentage of females captured is higher than for males during the fruit ripening period. Host plants, species that provide refuge for birds and water courses located at borders of the plot appear to be important factors in the spatial distribution of the pest and must always be considered when mass trapping is used.

13. Mass trapping technique using Maxitrap® trap, the attractant Ferag® CC 3D TM and the insecticide Ferag® ID TM (DDVP) is an effective method for the control *C. capitata* in peach orchards in the North East of Spain when the population level is low but when it is normal or high it must be reinforced by chemical spraying.

14. The proportion of traps to be checked is inversely related to the population density captured. It is not possible to reduce differentially the number of traps on the periphery and the inner part of the plot, or when the plot size is smaller than 1 ha. However for plots larger than 1 ha, it is enough to check 60% of the traps during the last 5 weeks of the ripening period, or 70% over the full period.