

8th International Symposium on Fruit Flies of Economic Importance



Valencia (Spain), 26 September - 1 October 2010

EDITORIAL
UNIVERSITAT POLITÈCNICA DE VALENCIA

8TH INTERNATIONAL SYMPOSIUM ON FRUIT FLIES OF ECONOMIC IMPORTANCE

VALENCIA, SPAIN



From 26th September to 1st October 2010

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Welcome message

On behalf of the Organizing Committee I wish to warmly welcome you to the 8th International Symposium on Fruit Flies of Economic Importance (ISFFEI 2010) in Valencia, Spain, from 26th September to 1st October 2010. I would like to thank to all those people who have contributed to the preparation of this Symposium as well as all the sponsors of this event.

We are living a very exciting scientific period, with considerable advances in the field of plant protection. The 8th ISFFEI symposium will reveal the increasing relevance of Tephritid research in the entire globe and the importance of fruit flies in world trade of fresh fruits. Recent genetic, biochemical, chemical, biological and microbiological breakthroughs have allowed new insights into the integrated fruit fly management programs. However, there is a constant need not only to keep aware of and develop new scientific approaches but also to identify adequate and effective environmentally safe control strategies for the different tephritids of economic importance. The members of the Organizing and Steering Committees have build a scientific program which will provide a balanced overview of advances in Tephritids' basic and applied research, with special focus on control programs.

The symposium venue is the Nexus building, the Congress centre of the Universidad Politécnic de Valencia, that will provide an appropriate and secure setting, with up-to-date technical facilities for the meeting. In addition to an exciting scientific program, you will benefit from the end of the summer, one of the nicest seasons in Valencia. Moreover, the cultural background and historical landscape that make of Valencia one of the most attractive cities in Europe, should contribute towards making this event an unforgettable moment. We hope that this unique environment will be a perfect time to meet friends and colleagues, and to establish or reinforce collaborations with peers from elsewhere.

Tephritid researchers from all over the globe have convened in Valencia and the 8th ISFFEI is already here. I expect that the symposium will be a great success and look forward to meeting all you during these days.

Alberto Urbaneja

8th ISFFEI Chairman

8th ISFFEI Committees

Organizing Committee

Chairman

Dr. Alberto Urbaneja García

Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA).

Secretary team

Dr. Beatriz Sabater-Muñoz (Centro de Protección Vegetal y Biotecnología, IVIA)

Ing. Rafael Argilés (Medfly SIT working program Spain - TRAGSA, IVIA)

Ms. Tania Navarro (Secretariat Fundación Agroalimed)

Members

Dr. Luis Navarro (Centro de Protección Vegetal y Biotecnología, IVIA)

Dr. Florentino Juste (Director IVIA)

Dr. Pedro Castañera (Centro de Investigaciones Biológicas, CIB CSIC)

Mr. Fernando Hernandez (Fundación Agroalimed)

Dr. Francisco Beitia (Centro de Protección Vegetal y Biotecnología, IVIA)

Dr. Josep A. Jacas (Departament de Ciències Agràries i del Medi Natural, Universidad Jaume I)

Dr. Ferran García Marí (Unidad de Entomología Agroforestal. Instituto Agroforestal Mediterráneo. Universidad Politécnica de Valencia (UPV).

Dr. Jaime Primo (Centro de Ecología Química Agrícola (CEQA), UPV)

Dr. Vicente Navarro (Centro de Ecología Química Agrícola (CEQA), UPV)

Dr. Joel González (CIB)

Staff

Dr. Cristian Vidal-Quist (Centro de Protección Vegetal y Biotecnología, IVIA)

Ing. María Juan-Blasco (Centro de Protección Vegetal y Biotecnología, IVIA)

Dr. César Monzó (Centro de Protección Vegetal y Biotecnología, IVIA)

Dr. Victoria San Andrés (Centro de Protección Vegetal y Biotecnología, IVIA)

Dr. Tatiana Pina (Departament de Ciències Agràries i del Medi Natural, UJI)

Staff for Technical visits

Jaime García de Oteyza (TRAGSA, Medfly mass rearing facility)

Igancio Pla (TRAGSA, Medfly & parasitoids releasing facility)

Miguel Ángel Utrillas (TRAGSA, Medfly & parasitoids releasing facility)
Marta Briasco García (TRAGSA, Medfly & parasitoids releasing facility)
Carles Tur Lahiguera (TRAGSA, Medfly & parasitoids releasing facility)
Teresa Navarro Navarro (TRAGSA, Medfly mass rearing facility)
Marta Martínez Gonzalvo (TRAGSA, Medfly & parasitoids releasing facility)
Antonio Polido (TRAGSA, Medfly mass rearing facility)
Luis Villanueva Gallent (TRAGSA, Medfly mass rearing facility)
Germán Ceballos Chan (TRAGSA, Medfly mass rearing facility)

Scientific Committee

Dr. Alberto Urbaneja
Dr. Beatriz Sabater-Muñoz
Dr. Vicente Navarro
Dr. Francisco Beitia
Dr. Josep Jacas
Dr. Ferran García Marí
Mr. Rafael Argilés
Dr. Brian Barnes
Dr. Anna Malacrida
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Dr. Nikos Kouloussis
Dr. Pablo Liedo
Dr. Aldo Malavasi
Dr. Robert Mangan
Dr. Pablo Montoya
Dr. Serge Quilici
Dr. Monica Spinetta
Dr. Roberto Zucchi
Dr. Kenneth Bloem
Dr. Nicanor Liquido

Fruit Fly Steering Committee (as in June 2008)

Chairman

Dr. Brian Barnes

ARC Infruitec-Nietvoorbij Fruit, Vine & Wine Institute, Stellenbosch

Members

Sunday Ekesi (International Centre of Insect Physiology and Ecology (*ICIPE*), Nairobi, KENYA)

Nancy Epsky (Subtropical Horticulture Research Station, USDA ARS, Miami, USA)

Yoav Gazit (The Israel Cohen Institute for Biological Control, PPMB - Citrus Division, Rishon Le-Zion, ISRAEL)

Nikos Kouloussis (Aristotle University of Thessaloniki, School of Agriculture, 54124 Thessaloniki, GREECE)

Pablo Liedo (El Colegio de la Frontera Sur (ECOSUR), Tapachula, Chiapas, MEXICO)

Anna Malacrida (Università di Pavia. Dipartimento di Biologia Animale, ITALY)

Aldo Malavasi (Moscamed Medfly Program, Sao Francisco Valley, Bahia, BRAZIL)

Robert Mangan (Crop Quality and Fruit Insect Research, USDA ARS, Weslaco, Texas)

Pablo Montoya (Programa Moscafrut, SAGARPA-IICA, Tapachula Chiapas, MEXICO)

Serge Quilici (CIRAD Réunion, Pôle de Protection des Plantes, La Réunion, FRANCE)

Pedro Rendon (USDA/APHIS/PPQ, Methods Development Section, Guatemala City)

Catherine Smallridge (South Australian Research and Development Institute, Adelaide, South Australia, AUSTRALIA)

Mónica Spinetta (Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA) de Argentina, Programa Nacional de Control y Erradicación de las Moscas de los Frutos, Buenos Aires, ARGENTINA)

Michael Stefan (Fruit fly Exclusion and Detection Programs, PPQ, APHIS, Riverdale)

Roberto Zucchi (University of Sao Paulo (USP/ESALQ), BRAZIL)

Acknowledgement to Official Organizing & Supporting Institutions

Organizing Institutions:

- Instituto Valenciano de Investigaciones Agrarias (IVIA)
- Universitat Jaume I (UJI)
- Universidad Politécnica de Valencia (UPV)
- The Fundación Agroalimed

The Organizing Committee would like to give thanks to the following Spanish and international institutions which had provided funds to develop this event:

- Generalitat Valenciana (GVA) project AORG/2010/031
- Instituto Nacional de Investigaciones y Tecnología Agraria y Alimentaria (INIA) project AC2009-00055-00-00
- Universidad Politécnica de Valencia (UPV) project 2473
- The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

Acknowledgement to Sponsors & Exhibitors

In the following pages delegates could read about our valuable sponsors and exhibitors.

Sponsors:

- Platinum: **SYNGENTA**
- Golden: **BAYER CROPSCIENCE, CERTIS** and **DOWAGROSCIENCES**
- Silver: **FRUIT FLY SERVICES SL.**
- Bronze: **BIAGRO SL.**

Exhibitors:

- AGRISENT
- BIOIBÉRICA
- COLTEC
- FRUIT FLY SERVICES SL.
- MUBARQUI Servicios Aéreos
- SCIENTIFIC ADVISORY SERVICES Pty. Ltd.
- SUTERRA
- SYNGENTA

The Organizing Committee would like to give all them the thanks for their support to this event.

Thank you!

About ISFFEI

Initiated in 1982, the quadrennial fruit fly symposium is being well established as a key meeting for bringing together scientists and fruit fly workers from all over the world.

The purpose of this Symposium is to bring together academic, industry, research and action people from the all over the world to discuss the various aspects of fruit flies and recent developments related to Pest Management Strategies.

The meeting will encourage cooperation between participants, contribute to spread new findings, promote their practical use and highlight research needs as it has been done in the previous symposia.

Past Symposia:

- The First International Symposium on Fruit Flies of Economic Importance, Athens, Greece, November 16-19, 1982.
- The Second International Symposium on Fruit Flies of Economic Importance, Crete, Greece, 16 - 21 September 1986.
- The Third International Symposium on Fruit Flies of Economic Importance, Antigua, Guatemala, 14 - 20 October 1990.
- The Fourth International Symposium on Fruit Flies of Economic Importance, Sand Key, Florida, USA, 5 - 10 June 1994.
- The Fifth International Symposium on Fruit Flies of Economic Importance, Penang, Malaysia, 1 - 5 June 1998.
- The Sixth International Symposium on Fruit Flies of Economic Importance, Stellenbosch, South Africa, 6 - 10 May 2002.
- The Seventh International Symposium on Fruit Flies of Economic Importance, Salvador, Bahia, Brazil, 10 - 15 September 2006.

General information

Congress language

The official language will be English. However, due to legal requirements, our government representatives will use one of the two official languages in Valencia, Spanish or Valencian with English simultaneous translation for the Opening ceremony.

Congress technical organizers

Grupo Pacifico

Paseo General Martínez Campos, 44, 1º

28010 Madrid - SPAIN

Tel. (34) 913.836.000 ext. 127

Fax. (34) 913.023.926

isffe2010@pacifico-meetings.com;

broca@pacifico-meetings.com

cherrera@pacifico-meetings.com;

Congress venue

The Nexus building (6G in the map) of the **Universidad Politécnica de Valencia** (UPV) harbors the Congress Centre of this Campus and will provide an appropriate and secure setting, with up-to-date technical facilities, to allow the perfect development of the Symposium program.

This building can be reached from entry "L" or "K", and is 1-2 minutes walking from **Galileo Galilei** Hall of Residence (6B in the map).

The tram is running all along the UPV Campus, with four stops marked with a white "m" in a red circle in the map. The nearest tram stop to the Symposium venues is "Tarongers" (which means orange trees in Valencian).

The UPV Campus map is on page 18

Registration desk & Technical secretariat

The 8th ISFFEI technical secretariat is formed by personnel of Grupo Pacifico. The technical secretariat will be open for on-site registrations and for information about the Symposium, at the Symposium hours.

The registration desk will be located in the main entrance hall level, please follow indications or ask Organizers Staff (easily to identify by their T-shirts and badges).

Internet access & Stations

All delegates will be provided with a personal key code (username and password) to access the wireless net. Internet stations and an area available to delegates to send and receive email messages free of charge will be located in the first floor.

Shuttle services & Public transport in Valencia city

A shuttle transport will operate between the appointed NH hotels and the ISFFEI venue as per the schedule on Program. Buses will depart twice in the morning and after all conferences with 20 minutes of difference between trips. More detailed timetable will be set up in the NH hotel hall.

Shuttle buses will stop at the "L" entrance of the UPV Campus.

Delegates hosted in other hotels are aimed to be in time in the NH hotel hall or have their own transfer to the UPV Campus.

Valencia city transport guides will be provided to all the delegates within the 8th ISFFEI bags and have been provided by the Valencia convention bureau. Other hotels have also information about public transport from any point of Valencia city to the ISFFEI venue.

Accompanying persons

Accompanying persons who are registered as such will receive a name badge and tickets for all the ceremonies. Accompanying persons holding the ID badges are entitled to travel on the official shuttle transportation, attend the opening ceremony, welcome reception and closing dinner, visit the exhibition hall and participate on the Accompanying Persons City Tour on Tuesday 28th September from 9:00 to 13:00.

City Tour will depart at 9:00 from NH Las Artes I hotel.

Speakers, audio visual & Technical desk

World class audio-visual and technical services will be provided by an expert team of UPV, which will be located in the main entrance hall level. Please, follow indications or ask Organizing Staff.

All presentations are to be computer generated, mainly with MS-PowerPoint (v2003-07) or compatible software, and provided as .ppt or .pps files. Speakers are required to report to this desk with their CD's, memory sticks (USB) or laptops with at least 24h prior to making their presentation. At Session breaks, presenters will be able to check the performance of their presentation with the hardware of the Auditorium.

First day presenters are encouraged to report to the centre well in advance of their presentation times to avoid queues and possible delays in the Symposium timetable.

All presentations should be uploaded to the Auditorium central computer. Own computers will not be admitted as source for the presentation in the Auditorium, the Organizer cannot guarantee projection of presentations that are handed in this way.

If you didn't submit a pdf copy of your talk by 24th September, please refer also to this desk to upload it for its inclusion in the USB key with all the information of the 8th ISFFEI. You can upload a slightly modified version of your talk protecting your valuable data, but please give the opportunity to all delegates to keep a copy of the main ideas of your work. This USB key will be distributed among all participants.

A presentation certificate will be delivered to the presenting delegate after talk in the Speaker Desk. Only one certificate, listing all the contributing authors, will be distributed for each talk.

Poster presenters

A Poster Information desk will be located within Poster room number one in the main entrance hall level. In this desk you will find your poster board number and double side tape to affix the poster to the poster board. Posters should be mounted on the poster boards early on Monday September 27th and will be hold during the all week. Posters must be removed on Thursday 30th September at 6 pm. Any poster not removed will be taken down by the Organizers and stored until Technical visit. Poster not collected during Technical visit will be discarded.

If you did not submit a pdf copy of your poster by 24th September, please refer to the audio-visual desk to upload it for its inclusion in the USB key with all the information of the 8th ISFFEI. This USB key will be distributed among all participants.

A presentation certificate will be delivered to the presenting delegate after poster holding in the Poster Desk. Only one certificate, listing all the contributing authors, will be distributed for each poster.

Abstracts & CD provided

All talk and poster abstracts are included in this book as well as in the CD within the delegate bag.

Special Events

Welcome Reception: Sunday 26th September at NH Las Artes I hotel

Opening Ceremony: Monday 27th September, at the Auditorium, by the Honourable Consellera of Agriculture of Valencia.

Closing Ceremony: Thursday 30th September, at the Auditorium.

Farewell dinner: Thursday 30th September, at the City of Arts & Science.

Technical visit: Friday 1st October, see schedule for further information.

Bids for the 9th ISFFEI: Tuesday & Wednesday, by the Steering Committee.

Coffe breaks & Lunch

Breaks are planned as in the 8th ISFFEI schedule and will take place in a big top tent-like structure located outdoors at 200 meters from the building entrance.

Morning coffee breaks will take place in this tent, whereas in the afternoon, a coffee station will be available in Poster room no.1 close to the Exhibition hall, just on the left side of the Auditorium.

Exhibition

The exhibition hall will be located in the main entrance hall level, and will be open in the same timetable as the Symposium.

A list of participating Exhibitors appears in the following pages of this book and all delegates are encouraged to visit them.

SPONSORS



	Línies <i>Líneas</i> Routes 18-29-30-40-41-71		Línies <i>Líneas</i> Routes 18-29-30-40-41-71		Línies <i>Líneas</i> Routes 9-40-41-71-18-29-30		Línies <i>Líneas</i> Routes 9-30-40-41-71
	Línies <i>Líneas</i> Routes 30-40-41-71		Línies <i>Líneas</i> Routes 18-29		Línia <i>Línea</i> Route 1		
	Porta d'accés <i>Puerta de acceso</i> Entrance gateway		Porta d'accés <i>Puerta de acceso</i> Entrance gateway		Entrada a l'edifici <i>Entrada al edificio</i> Entrance to building		Entrada a l'edifici <i>Entrada al edificio</i> Entrance to building
	Punt de trobada <i>Punto de encuentro</i> Meeting point		Punt de trobada <i>Punto de encuentro</i> Meeting point		Caixer automàtic <i>Caixer automático</i> Cash point		Caixer automàtic <i>Caixer automático</i> Cash point
	Informació <i>Información</i> Information		Informació <i>Información</i> Information		Servei mèdic <i>Servicio médico</i> Health centre		Servei mèdic <i>Servicio médico</i> Health centre
	Parada de metro <i>Parada de metro</i> Railway stop		Parada de metro <i>Parada de metro</i> Railway stop		Quiosco de premsa <i>Quiosco de prensa</i> News-stand		Quiosco de premsa <i>Quiosco de prensa</i> News-stand
	Parada de metro <i>Parada de metro</i> Railway stop		Parada de metro <i>Parada de metro</i> Railway stop		Escola infantil <i>Escuela infantil</i> Nursery		Escola infantil <i>Escuela infantil</i> Nursery
	Parada de metro <i>Parada de metro</i> Railway stop		Parada de metro <i>Parada de metro</i> Railway stop		Piscina coberta <i>Piscina cubierta</i> Indoor pool		Piscina coberta <i>Piscina cubierta</i> Indoor pool
	Parada de metro <i>Parada de metro</i> Railway stop		Parada de metro <i>Parada de metro</i> Railway stop		Aparcament <i>Aparcamiento</i> Parking		Aparcament <i>Aparcamiento</i> Parking
	Parada de metro <i>Parada de metro</i> Railway stop		Parada de metro <i>Parada de metro</i> Railway stop		Servei de seguretat <i>Servicio de seguridad</i> Security service		Servei de seguretat <i>Servicio de seguridad</i> Security service
	Parada de metro <i>Parada de metro</i> Railway stop		Parada de metro <i>Parada de metro</i> Railway stop		Cafeteria <i>Cafetería</i> Cafeteria		Cafeteria <i>Cafetería</i> Cafeteria

Syngenta Crop Protection

Bringing plant potential to life

We bring plant potential to life

Syngenta is one of the world's leading companies with more than 25,000 employees in over 90 countries dedicated to our purpose: Bringing plant potential to life.

Our Crop Protection and Seeds products help growers increase crop yields and productivity. We contribute to meeting the growing global demand for food, feed and fuel and are committed to protecting the environment, promoting health and improving the quality of life.

Syngenta offers a uniquely broad product portfolio leader in crop protection and high-value commercial seeds, World-class science (leader in research and development with \$1 billion R&D investments in 2009 and 5,000 people in R&D around the world), global reach and experience (over 25,000 employees in more than 90 countries) and commitment to working with customers (tailoring solutions to individual needs).

Major product brands include Herbicides control weeds that compete with crops for light and nutrients, Fungicides to prevent and cure fungal disease, Insecticides to control pests which reduce yields by damaging crops, Seed Care products for protecting young plants against insects and disease and Seeds for corn, soybean, sugar beet, cereals, oil crops; vegetables and flowers.

Since 2006, **Syngenta** commercializes in Spain and other Mediterranean countries Adress[®], a new method to control Med Fly based in the reduction of fly populations. Adress[®] system was developed together by Syngenta and the University of Valencia helps farmers to control this important pest with technology respectful with the environment and beneficial insects and it is perfectly compatible with other methods such as sterile insect technique (SIT).

Adress[®] has been tested in many trials and different conditions in citrus, top fruits, table grapes and tropical fruits, including the complete protection of the whole Hierro Island (Canary Islands).

For more information please visit:

www.syngenta.com



Bayer CropScience

Bayer CropScience aims to contribute to sustainable agriculture, which means achieving a harmonious balance between economic success, environmental responsibility and social acceptability.

With its considerable investment in R&D, **Bayer CropScience** is able to offer technologies and agricultural solutions that guarantee crops, reduce losses, improve the quality of produce from the fields and optimise the use of natural resources, contributing to meeting world food and animal feed requirements.

Our company is one of the global leaders in the supply of innovative products, not only of insecticides and herbicides, fungicides and seed treatments but also of integral solutions and services.

With respect to insecticides, **Bayer CropScience** has always had a high profile in the control of *Ceratitis capitata*, being the company that developed **Fention** (Lebaycid) that made it possible to control this pest for many years. Our company continues to conduct research to develop new solutions that will meet the demands of sustainable agriculture, in agreement with the demands we place on ourselves.

In line with this strategy, **Bayer CropScience** will be launching an innovative solution as part of its Mass Trapping solutions this year. This is **Decis Trap**, which will enable us to contribute to the necessary modernisation required by all the *Ceratitis* control systems. We are also taking initial steps in cooperation with various official bodies, to conduct research into new solutions that will help to provide an integrated response for the correct management of this pest, which is of recognised importance to agriculture in the Mediterranean area.

For more information please visit:

<http://www.bayercropscience.es/>

<http://www.bayercropscience.com/>

CERTIS

The logo for CERTIS IPM Solutions features the word "CERTIS" in a large, blue, serif font. A green diagonal line starts from the bottom left of the "I" in "CERTIS" and extends upwards and to the right, crossing the "S". Below "CERTIS" is a horizontal green line. Underneath this line, the words "IPM Solutions" are written in a blue, sans-serif font. The "I" in "IPM" is partially covered by the green diagonal line.

IPM Solutions

Since its launch in 2001, **Certis Europe** has developed and grown steadily. It has expanded its activities both geographically and in terms of technologies and product portfolio. Unique among suppliers to the horticultural and specialty crops sectors, **Certis** holds true to its original objectives to provide solutions for growers that take account of market demands and pressures, as well as crop production challenges.

Constantly at the forefront of innovation, **Certis Europe** uses a number of new technologies in its product development to promote sustainability. Important amongst these are the beneficial insects, including predators and parasitoids, biopesticides and other compatible products used in Integrated Pest Management (IPM) programmes to provide effective and sustainable pest control.

Virus-, bacteria- and pheromone-based products also offer important additions to the armoury and have proved a valuable asset in the development of integrated control strategies in both fruit and vegetables. A major advantage for all these products is that they leave no residues in fruit and have a zero harvest interval. **Certis** can thus provide growers with solutions to produce healthy, clean and safe crops, with protection available right up to harvest.

For more information please visit:

<http://www.certiseurope.com/>



Ceratitis capitata (Wiedemann), courtesy of USDA-ARS

GF-120[®] Fruit Fly Bait.

- Manage and eradicate populations of fruit flies quickly and easily using GF-120[®].
- GF-120[®] is a fruit fly bait consisting of natural feeding attractants with the active ingredient spinosad.
- All ingredients are optimized for attraction of multiple tephritid species including: Mediterranean fruit fly, melon fruit fly, Oriental fruit fly, olive fruit fly, cherry fruit fly, walnut husk fly
- Attract and control male and female of major fruit fly species of economic importance.
- It has been used globally to eradicate and/or manage multiple species of fruit flies
- Does not disrupt beneficial insect populations
- Registered to be used in organic agriculture
- For more information please visit: <http://www.dowagro.com>





FRUIT FLY SERVICES, SL born as result of the union of two pioneer companies in rational control of agriculture pests by means of respectful systems with the environment, **PROBODELT** and **OPENNATUR**.

It born with the intention to become a referent in pest control of different dipterans pests that affect nowadays at Spanish crops, as well as new species that could introduced and could be potentially harmful to our crops.

PROBODELT, with a wide experience in pest control, was created with the target of design and execute rational pest control programmes based in integration of different techniques with easy application systems. Has been pioneer in introduction of mass trapping technique at commercial level against rice borer (*Chilo suppressalis*). It's specialized in insect traps manufacturing and patents makes it a reference in pest control sector.

OPENNATUR, specialized in pest control by means environmentally respectful methods, was created as well as **PROBODELT** with the target of design and execute pest control programmes. Since the beginning, its bet was strong in I+D+I purposes, its allows to be a reference in the pest control sector with support of other different techniques as monitoring, mass trapping, and mating disruption.

FRUIT FLY SERVICES unite in only one company the experience, knowledge and resources that had allows in a short period of time to become a reference company for pest control of *Ceratitis capitata* and *Bactrocera oleae*.

For more information please visit:

<http://www.opennatur.com/>

www.probodelt.com



BIAGRO S.L. is a Spanish company operating in Vegetable Nutrition and Integrated Pest Management. Founded by Mr. Antonio Quesada in 1982, the company started operating as a consultant of big fruits and vegetables producers with a branch society called Consultant Centre of Plant Nutrition, whose function was the nutritional diagnosis, fertilisation programs, observation and checking of agricultural industries and advice on growing techniques. After 16 years of operation, **BIAGRO SL.** produces its own fertilizers, correctors and natural fungicides, and distributes insect pheromones and IPM products from a well know suppliers. **BIAGRO S.L.** believes in continuous and accelerated growth through its own I+D department and through strategic research alliances with academic partnerships. At the same time, the Company is involved and cooperate with the Ministry of Agriculture in scientific programs to develop new IPM systems with pheromones and natural products.

BIAGRO SL. is a charter member of AEFA (Spanish Manufacturer Agronutrients Association). It forces us to make high quality products, adjusted to the Spanish and European rules. **BIAGRO SL.** has achieved the quality procedure ISO 9001.

Recently and regarding IPM of Tephritids in Spain BIAGRO SL. has developed **STARCE** a liquid bait with very interesting results (80% of medfly females captures vs 20% of males), and continues with the distribution of **M3 traps** (Green Trading Co.) for lure and kill medfly. M3 traps are being recommended by the Department of Agriculture of the Valencia Region Government.



Learn more at:

www.biagro.es



EXHIBITORS

List of Exhibitors:

- **AGRISENT**
- **BIOIBÉRICA**
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Agrisent de México SA de CV, fue incorporada en 1992. Líder en el suministro de ingredientes para dietas larvarias (harita de olote, levadura de torula), materiales y equipos para los Mercado de insectos benéficos y Sistemas Administrativos Integrados para el control y erradicación de plagas a Agencias inter-nacionales y Agrícolas gubernamentales federales, estatales y al sector privado en apoyo al esfuerzo, al estudio, desarrollo y producción de insectos benéficos. En cooperación con algunas agencias estatales Mexicanas y SAGARPA, ha desarrollado y probado, nuevos productos para el monitoreo y control de insectos. El más nuevo es el ECO-TML Phermone Plug, (Capsula de Trimedlure Ecológica).





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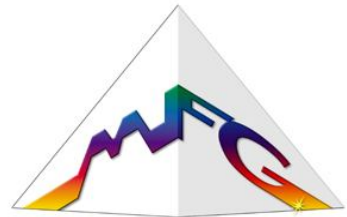
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Information: SAS has published a number of high quality wall posters on fruit flies (Tephritidae) of Australia and the World, as well as bell injection of bananas and insect pests and beneficials of bananas and papaws in Australia. These posters are valuable aids for primary producers as well as government quarantine agencies both in Australia and overseas. Scientific Advisory Services has recently formed a unique partnership with Starr, an exciting young Brisbane artist to produce a unique range of insect paintings.

SAS offers also insects embed into resin, as key rings. Please have a look in the trials with fruitflies as paper-holders just made a few weeks ago!

Some of the Starr insect paintings, beautiful!



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Information: **Suterra** is dedicated to aiding farmers in finding workable solutions by researching, developing and commercializing alternative IPM solutions using naturally occurring attractants and semiochemical compounds to protect crops such as pome fruit, stone fruit, citrus and vegetables from insect attack. Headquartered in Bend, Oregon and with regional offices in Spain, Italy, Mexico and Argentina, Suterra is a market leader in the manufacture and supply of a broad range of IPM tools for a variety of crop pests. Suterra's pheromone and attractant based control products are already registered in many global markets in the Americas, Europe and the Southern Hemisphere and Suterra's reputation and expertise is recognized throughout the world by university entomologists, government agencies and farming organizations. For fruit flies, the range extends beyond attractants for monitoring males and females and now includes effective attractant solutions for use in area-wide, mass trapping control campaigns and the novel attract and kill **Magnet™** tools for both Olive Fly and Mediterranean Fruit Fly: **Magnet™ OLI** and **Magnet™ MED**

Magnet™ MED



Program at glance

	26/09/10	27/09/10	28/09/10	29/09/10	30/09/10	01/10/10	
	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	
08.00		Registration and Poster holding					
08.30			Plenary conferences				Exit from hotels
09.00		Opening ceremony					
09.30		Plenary conference	Session 8	Session 7	Session 4	Technical visit SIT facilities & IVIA	
10.00							
10.30		Coffee break / Poster session					
11.00							
11.30		Session 3	Session 8	Session 7	Session 5		
12.00							
12.30							
13.00		Lunch					
13.30							
14.00							
14.30		Coffee break / Poster session	Coffee break / Poster session				
15.00							
15.30		Session 6 / Workshop	Session 1	Session 2	Session 5		
16.00			Break	General discussion	General discussion		
16.30							
17.00	Registration to the 8th ISFFEI at NH Las Artes I hotel	General discussion	Session 1	Meeting of TEAM	Closing session		
17.30							
18.00		General discussion	General discussion		Poster removal	Transfer to hotels	
18.30							
19.00							
19.30							
20.00	Welcome reception at NH Las Artes I hotel						
20.30							
21.00					Farewell dinner		
21.30							

Sessions

Session 1: Biology, Ecology & Behaviour

Session 2: Morphology & Taxonomy

Session 3: Genetics & Evolution

Session 4: Risk Assessment, Quarantine & Post-harvest Treatments

Session 5: SIT Principles & Applications

Session 6: Area-wide & Action Programs

Session 7: Natural Enemies & BioControl

Session 8: Chemical Ecology, Attractants & other Control Methods

Plenary Sessions

Monday 27th

Insecticide resistance in fruit flies: the case of malathion resistance in Spanish populations of *Ceratitis capitata* by Dr. Félix Ortego (CIB CSIC, Spain)

Tuesday 28th

A review of recent advances and future prospects in the study of fruit fly (Diptera: Tephritidae) - host plant relationships by Dr. Martin Aluja (Instituto de Ecología, Mexico)

Wednesday 29th

Fruit fly semiochemicals: 'Discovery and application of attractants and pheromones in fruit fly control methods' by Dr. Eric Jang (U.S. Pacific Basin Agricultural Research Center, USDA-ARS, USA)

Thursday 30th

In and out of Africa: invasion history of fruit flies on the African mainland (diptera, tephritidae) by Dr. M. de Meyer (Royal Museum for Central Africa (RMCA), Entomology Section, Belgium)

PLENARY TALKS

INSECTICIDE RESISTANCE IN FRUIT FLIES: THE CASE OF MALATHION RESISTANCE IN SPANISH POPULATIONS OF *CERATITIS CAPITATA*.

Ortego, Félix*; Couso-Ferrer, Francisco; Aroui, Rabeh; Perera, Nathalie; Beroiz, Beatriz; Cervera, Amelia; Hernández-Crespo, Pedro & Castañera, Pedro

Centro de Investigaciones Biológicas (CIB) del Consejo Superior de Investigaciones Científicas (CSIC), Departamento de Biología Medioambiental, Madrid, Spain. Email: ortego@cib.csic.es

Fruit flies are considered pests of major economic importance worldwide. A wide range of management strategies are being implemented for the control of these species, but most control programs depend on the use of insecticides. Recent studies have reported resistance in field populations of some species, such as the Mediterranean fruit fly (medfly) *Ceratitidis capitata* (Wiedemann), the oriental fruit fly *Bactrocera dorsalis* (Hendel), and the olive fly *Bactrocera oleae* (Gmelin). The establishment of resistant populations may become an important economic problem, particularly if cross resistance is detected in areas where pesticide application is restricted to a very few active ingredients. The identification of the mechanisms associated to resistance and the development of tools for the molecular or biochemical detection will be a key issue to be addressed in order to implement appropriate resistance management strategies counteracting the emergence and dispersal of resistance.

Resistance to malathion in field populations of *C. capitata* was first reported by our group in the Comunidad Valenciana (Spain), where an intensive use of this insecticide was maintained for several years. A point mutation of the acetylcholinesterase gene (*ace2*) was elucidated as the main resistance mechanism, though metabolic resistance mediated by esterases may also be involved. We have developed a rapid and simple PCR-RFLP diagnostic method for detection of malathion resistance alleles. Analysis of field populations from different geographical areas revealed that the resistant allele is widespread all over Spain, whereas it was not detected in populations from other countries.

Current medfly control in citrus orchards in Spain is mainly based on the use of spinosad and lambda-cyhalothrin, since malathion was disallowed in the European Union in 2009. The susceptibility of field populations to these insecticides and the cross-resistance exhibited by the malathion resistant strain is being evaluated. *C. capitata* populations tested so far have been highly susceptible to spinosad. However, remarkable differences in susceptibility to lambda-cyhalothrin have been found when comparing laboratory and field populations. In addition, selection for resistance to lambda-cyhalothrin or spinosad performed in the laboratory has resulted in a decreased susceptibility for both insecticides. The mechanisms underlying this resistance are under study.

Keywords: Medfly, malathion, cross-resistance, lambda-cyhalothrin, spinosad, esterases.

A REVIEW OF RECENT ADVANCES AND FUTURE PROSPECTS IN THE STUDY OF FRUIT FLY (DIPTERA: TEPHRITIDAE) - HOST PLANT RELATIONSHIPS

Aluja, Martín*; Birke, Andrea & Guillén, Larissa.

Instituto de Ecología, A.C. Xalapa, Veracruz, Mexico;. Email: martin.aluja@inecol.edu.mx

We provide an overview of recent advances in the study of fruit fly (Diptera: Tephritidae) - host plant relationships as well as a look into the future of this fascinating research area of great basic and applied interest. We start by reviewing the evolutionary, ecological, physiological and behavioral mechanisms related to host finding and utilization. We then review recent work by us and various other groups worldwide on host plant use patterns, dwelling on host resistance, fitness costs to the herbivore and the effect of the host on nutritional and immune reserves and sexual performance of adult flies. Examples used stem from work with *Anastrepha ludens*, *A. striata*, *A. fraterculus*, *Rhagoletis completa* and *Ceratitis capitata*, among others. We also discuss recent work on host effects on the next trophic level, using native and exotic braconid parasitoids such as *Utetes anastrephae* and *Diachamismorpha longicaudata* as models to illustrate the phenomenon. Along the way we discuss the great potential of new research tools such as proteomics in elucidating the fine details of host plant use by true fruit flies. We finish by discussing recent advances in host plant status designation; among them the process of determination of the fundamental and realized host ranges which impinge directly on regulatory procedures related to the export of fresh fruit worldwide.

FRUIT FLY SEMIOCHEMICALS: DISCOVERY AND APPLICATION OF ATTRACTANTS AND PHEROMONES IN FRUIT FLY CONTROL METHODS

Jang, Eric.

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Tephritid fruit flies are some of the most economically important pests of fruits and vegetables worldwide. As such the discovery and application of semiochemical attractants for detection and control have had a long history. Early attractants were “discovered” quite by chance but was quickly followed by both empirical and non-empirical research that lead to the identification of pheromones, kairomones for some flies. True pheromones have played only a minor role in control while the “parapheromones” have played a major role, especially for flies in the family *Bactrocera*. Fruit flies in the family *Anastrepha*, *Rhagoletis*, and *Ceratitis* have depended primarily on the application of proteinaceous food-based lures. This is also true of most of the other (non-*Bactrocera*) tephritids. Recently, improvements in food-based lures have resulted in improvements in the application of such attractants for some of the above species.

The most successful example of the use of attractants for control involves the use of the parapheromone methyl eugenol for the control of certain *Bactrocera* fruit flies such as the oriental fruit fly, *B. dorsalis*. This technique coined male annihilation or MAT has been the centerpiece of semiochemical-based control and eradication technologies.

Interestingly behaviorally based methods such as “pheromone disruption” has not been developed to date; perhaps due to the lack of truly powerful pheromones identified for this genera. Much of the current research has focused on improvements in attractants through chemical structure-activity type screening that as resulted in some improvements and well as a host of new host-based kairomone attractants. Formulation of multiple-component attractants continues to be a challenge but promise to further improve on the use of semiochemical attracts for detection and/or control.

IN AND OUT OF AFRICA: INVASION HISTORY OF FRUIT FLIES ON THE AFRICAN MAINLAND (DIPTERA, TEPHRITIDAE).

De Meyer, Marc^{*1}; Ekesi, Sunday²; Virgilio, Massimiliano¹⁻³; Khamis, Fathiya²; Mwatwala, Maulid⁴ & Malacrida, Anna⁵

¹Royal Museum for Central Africa (RMCA), Entomology Section, Tervuren, Belgium; Email: marc.de.meyer@africamuseum.be; ²International Centre for Insect Physiology and Ecology, Nairobi, Kenya; ³Royal Belgian Institute of Natural Sciences, Invertebrates Section, Brussels, Belgium; ⁴Sokoine University of Agriculture, Department of Crop Science and Production, Morogoro, Tanzania; ⁵Pavia University, Department of Animal Biology, Pavia, Italy

Recent increases in trans-continental transport of tropical fruits and vegetables as a result of globalization in trade, as well as human traffic either for leisure (tourism) or business, is leading to the increased risk of inadvertently introducing alien invasive species to countries or regions where they not already occur with devastating economic impact if not controlled. Such human activities cause dispersal of organisms over long distances that could not be covered by natural means. The introduction of species into a new area can alter successional patterns, mutualistic relationships, community dynamics, ecosystem function, and resource distribution. Fruit flies (Diptera, Tephritidae) are a group of organisms that are very susceptible to such human induced introductions and few insects have greater impact on international marketing and world trade in agricultural produce than tephritid fruit flies. As important pest species of several horticultural products like fruits and vegetables, migration and transport of horticultural produce has lead to (pre-) historic cases of accidental introductions. Although Africa is known to be the origin of several fruit fly introductions and establishments worldwide (the most notorious species being the Mediterranean fruit fly, *Ceratitis capitata*), with the intensification of fruit trade, the continent has also become highly vulnerable to introduction of alien fruit fly species. Notable invasive species include *Bactrocera zonata*, *Bactrocera invadens*, *Bactrocera latifrons* and *Bactrocera cucurbitae*.

Lately, molecular methodologies is providing useful tools in detecting and analysing alien introductions. DNA barcoding, using a short gene sequence taken from standardized portions of the genome, allow recognition of indigenous and alien species even in immature stages that are inseparable on morphological grounds. This allows quarantine officers and national plant protection agencies to identify intercepted material during inspections or surveying activities. Molecular phylogeny indicates the lineages among species, recognizing clusters of closely related taxa. Combined with host plant data, patterns in host-insect relationships can be revealed. Population genetics can provide an insight in the macrogeographic population structure and interrelationships between regions, indicating possible sources of introductions or migrations. Molecular phylogeography allows a reconstruction of pathways of geographic range expansion. The application of molecular tools in these fields is demonstrated here with some examples of fruit fly pests originating from or introduced to the African mainland.

ORAL PRESENTATIONS

Session 1
Biology, Ecology & Behaviour

WOLBACHIA IN EUROPEAN, PERSIAN AND RUSSIAN *RHAGOLETIS CERASI* (DIPTERA; TEPHRITIDAE) POPULATIONS.

Rasool, Bilal^{1,2*}; Arthofer, Wolfgang³; Schuler, Hannes²; Riegler, Markus⁴; Mirak, Tohid N⁵. & Stauffer, Christian²

¹Nuclear Institute for Agriculture & Biology, Faisalabad, Pakistan, bilalisb2001@yahoo.com; ²Institute of Forest Entomology, Boku, University of Natural Resources & Applied Life Sciences, Vienna, Austria. ³Molecular Ecology Group, University of Innsbruck, Austria. ⁴Centre for Plants and the Environment, University of Western Sydney, Australia. ⁵Cereal Research Department; Karaj, Iran

Background: The European Cherry Fruit Fly, *Rhagoletis cerasi*, is an established field model species for multiple *Wolbachia* infections and cytoplasmatic incompatibility CI. European populations of *R. cerasi* were described to be infected with at least five *Wolbachia* strains *wCer1-5* and *wCer2* causes almost complete CI between infected males and uninfected females. We will compare the *wCer* infection between *R. cerasi* specimens collected in 2002 and those collected in 2009. In 2009, also specimens from Persia – the centre of origin of the host tree *Prunus* spp. – and from Central Russia were analysed.

Methods: Pupae were collected in diverse areas of Europe, one location close to Teheran (Iran) and one location in Tomsk (Russia). Specimens were analyzed by sequencing *wsp* amplicons. Southern hybridization technique was applied for the detection of the recombinant low titre strain *wCer3*.

Results: *wCer1* was fixed in all populations whereas *wCer2* was present only in South and Central Europe. The *wCer2* spread in Europe from South to North had not changed much from 2002 to 2009 confirming the low migration rate of *R. cerasi*. The abundance of *wCer4* was homogenous across Iran and Russia and the frequency of *wCer5* was highest in the Persian population. *wCer5* showed significant differences in spatial distribution.

Conclusions: No additional *wCer* strains were detected in Persia. Persia is described to be the center of origin for *Prunus* spp.. These centers are assumed to comprise the highest biodiversity and also genetic diversity. As *wCer2* was not detected in Persia, a recent spread of *wCer2* from South Europe is likely. Considering the low migration of *R. cerasi* and the presence of *wCer1,4,5* in Iran and in the remote and isolated Russian area it is likely that these strains originate from Persia. From there, *R. cerasi* spread parallel to its host tree into the Palearctic region.

Keywords: *Rhagoletis cerasi*, *Wolbachia*, cytoplasmatic incompatibility, *Prunus*, distribution

INTRA AND INTERSPECIFIC LARVAL COMPETITION BETWEEN *CERATITIS CAPITATA* AND *ANASTREPHA FRATERCULUS*.

Liendo, M. Clara^{*1,3}; Devescovi, Francisco^{1,3}; Parreño, María A.¹; Milla, Fabián H.¹; Carabajal Paladino, Leonela^{1,3}; Vera, M. Teresa^{2,3}; Segura, Diego F.^{1,3} & Cladera, Jorge L.¹

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Background: *Ceratitidis capitata* and *Anastrepha fraterculus* are two major fruit pests in Argentina where they are commonly found sharing its host fruit with conspecific and heterospecific larvae. As larvae are incapable of leaving the fruit, they are forced to share nutritional resources, and eventually compete for them with other larvae. Larval competition can occur by direct interference (contest) or exploitation (scramble). The impact of competition will depend both on the density of larvae within a particular fruit and the relative abundance of each species. Here we studied both the effects of competing with conspecific and heterospecific larvae for *A. fraterculus* and *C. capitata*.

Methods: Intraspecific competition was analyzed by transferring newly hatched larvae into a fixed amount of larval diet at increasing densities. Interspecific competition was analyzed by keeping total density constant and transferring different proportions of larvae of the two species. We measured percentage of recovered pupae and adults, pupae weight, and larva-pupa and larva-adult developmental times.

Results: Under intraspecific competition, *C. capitata* showed a density-dependent reduction in pupae weight and percentage of recovered pupae. *A. fraterculus* showed also a density dependent effect on pupae weight, and a decrease in larva-pupa developmental time. Comparing the two species, in *C. capitata* the reduction in pupae weight was more homogeneous among individuals, whereas in *A. fraterculus* a majority of pupae showed a marked decrease in weight and only few pupae showed slight negative effects. Under interspecific competition, we found that when the relative density of *A. fraterculus* larvae increased, the percentage of recovered pupae and the pupae weight increased for *C. capitata*. Likewise, *A. fraterculus* pupae weight increased when the relative density of *C. capitata* increased.

Conclusions: Our results suggest that *C. capitata* larvae facing a shortage of resources develop a strategy of resource distribution similar to that described as scramble competition, because the individuals showed a homogeneous impact on their weight. Conversely, *A. fraterculus* larvae showed a response similar to that described as contest competition, because as the number of individuals in the container increases, the negative effect is not the same for every individual. Finally, we found that *C. capitata* and *A. fraterculus* larvae suffer more from sharing the resource with conspecific larvae than heterospecific larvae.

Keywords: *Ceratitidis capitata*, *Anastrepha fraterculus*, interspecific competition, intraspecific competition, larval competition.

EFFECTS OF INTRASPECIFIC COMPETITION ON THE LARVAL DEVELOPMENT AND PUPAL WEIGHT OF *DACINI* (DIPTERA: TEPHRITIDAE) INFESTING CUCURBITS IN LA RÉUNION.

Jacquard, Cathy*; Deguine, Jean-Philippe; Delatte, Hélène; Payet, Christine & Quilici, Serge

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Background: In La Réunion, cucurbit crops suffer considerable damage due to fruit fly attacks. A complex of three species (The Melon fly, *Bactrocera cucurbitae*, The Ethiopian fly, *Dacus ciliatus*, and the Indian Ocean fruit fly, *Dacus demmerezi*) coexist in the island and can infest 16 different species of Cucurbitaceae. In order to develop sound and sustainable management methods for these pests, we need to know more about their behavior and ecology. The objective of this study is to clarify the interaction existing at larval stage, and particularly the competition which may occur between conspecific larvae in a same fruit. According to earlier studies, squash (*Cucurbita pepo*) was chosen for laboratory experiments because it is one of the most damaged cultivated cucurbit in La Réunion.

Method: To measure the effect of intraspecific competition on the survivorship rate of larvae and on the pupal mass, infestations of squash were carried out in the laboratory with L1 larvae of *B. cucurbitae*, *D. ciliatus* or *D. demmerezi*, obtained from lab rearing.

Five levels of infestation were selected e.g. A) One larva (L1) for two grams of squash, B) One larva/1 g, C) Two larvae/1 g, D) Four larvae/1 g and E) Eight larvae/1 g. Each level was replicated five times. Four days after infestation, pupae were collected daily, weighed individually and grouped by weight classes in a same box and when they emerged, the adults were sexed.

Results: Larvae of *B. cucurbitae* and *D. demmerezi* don't have the same response to an increase of intraspecific competition in a same fruit. Larvae of the first species show a good survivorship level (63% for level D) whereas larvae of *D. demmerezi* are more affected by a high level of competition (18% for level C). On the contrary, pupal weight of *B. cucurbitae* clearly decreases when the number of larvae increases in the fruit while pupal weight of *D. demmerezi*, does not seem to be affected by intraspecific competition.

Conclusion: It seems that, in the complex of Dacini attacking cucurbits, the species do not have the same strategy regarding intraspecific competition. The larvae of *B. cucurbitae* are able to share resources and maintain a good survivorship finally giving more adults with low weight. Conversely, in *D. demmerezi* larval survivorship is decreased but the pupal weight of survivors remains high. Further studies will be needed on larval interspecific competition in this complex of fruit flies, in order to understand the performance of each species in situations of co-infestation.

Keywords: larval intraspecific competition, La Réunion, Dacini, *Cucurbita pepo*

OVIPOSITION CHOICE OF *BACTROCERA PAPAYAE* DREW & HANCOCK (DIPTERA: TEPHRITIDAE): FULLY-RIPE FRUIT OR UNRIPE FRUIT WITH WOUND ON FRUIT SURFACE.

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Background: The oviposition preference of many species of tephritid fruit fly relies on fruit firmness. To minimise aculeus wear, female tephritid flies are thought to prefer to oviposit in fruit wounds or softer exopericarp of ripe and fully-ripe fruits over harder exopericarp of unripe fruit. The fully-ripe fruit was suitable for larval growth and preferred for oviposition by female fly of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) more than unripe fruit, soft exocarp of fully-ripe fruit appeared to be the limiting factor of oviposition, with few successful ovipositions recorded in all studies, and very few in unripe fruit. In this study, we asked whether female flies that were exposed to both fully-ripe fruit without wound and unripe fruit with wound on fruit surface, would choose fully-ripe fruit for oviposition or not. The result of this study can enhance our understanding of host utilization by female fly.

Methods: A choice experiment was conducted to determine the behavior of individual female *Bactrocera papayae* Drew & Hancock (Diptera: Tephritidae). Both fully-ripe mango without wound and unripe mango with wound on fruit surface of mango, *Mangifera indica*, Namdorkmai variety (Anacardiaceae) were offered simultaneously in a 30×30×30 cm observation cage. An individual, 21–22-day-old, mated female fly was released in an observation cage. Fruit fly behaviors observed and recorded were: (i) duration of fly visits to a fruit, (ii) number of attempted ovipositions (unsuccessful penetration), and (iii) duration of successful oviposition events. Observations were done from 09:00–15:00 hours. Twenty single-fly replicates were conducted. At the end of the day, female flies were dissected to check whether eggs were presented in their ovaries. All fruits that female flies had laid eggs into were dissected for number of egg count.

Results: The total duration of visits differed significantly between the stages of ripening (t-test: $t = 15.010$, d.f. = 38, $P < 0.0001$), with unripe fruits being visited in longer and fully-ripe fruit significantly less. There were also significant differences in the number of attempted ovipositions between the various fruit ripening stages, with fewer attempted ovipositions into fully-ripe fruit and higher penetration into unripe fruit (t-test: $t = 6.696$, d.f. = 38, $P < 0.0001$). All female flies could not oviposit into full-ripe fruits. The average time of successfully oviposition duration into wound on unripe fruit of female flies is 35.05 ± 1.52 minutes.

Conclusions: The results of this study indicated that fruit wound play an important role in the oviposition determination of female fly *B. papayae*. Female *B. papayae* chose unsuitable host that she could easier oviposit than nutritious host which was hard to oviposit. Thus, it is difficult to assume that oviposition preference of female flies relates solely to the performance of their larvae.

Keywords: *Bactrocera papayae*, oviposition preference, mango, ripening.

PHENOTYPIC PLASTICITY OF THERMAL TOLERANCE IN ADULT *CERATITIS CAPITATA* AND *CERATITIS ROSA* (DIPTERA: TEPHRITIDAE).

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Background: The invasion success of *Ceratitis capitata* relative to its congener, *Ceratitis rosa* probably stems from physiological, morphological and behavioural adaptations which enable them to survive in different habitats. However, few studies have investigated thermal tolerance as a means to explain biogeographic differences or likely invasion potential. Here we report on two main questions. First, we ask if *C. capitata* is aided during the invasion process by phenotypic plasticity of thermal tolerance relative to a narrowly-distributed congener *C. rosa*. Second, we investigated thermal tolerance and their responses to temperature acclimation conditions in both species.

Methods: First, we examined rapid cold hardening (RCH) and rapid heat hardening (RHH) in *C. rosa* and *C. capitata* by investigating the effects of sub-lethal low and high temperature pretreatments on low and high temperature survival respectively. Since RCH was more pronounced than RHH, we then further examined the time course of RCH. Using a population extinction model in various thermal habitat scenarios, coupled with microclimate data where both species cohabit, we then estimated the potential fitness effects that variation in RCH might afford *C. capitata* under natural conditions. Second, using a full-factorial design, the responses of upper and lower critical thermal limits (CTLs) to one week of acclimation at three mild temperatures (20, 25 and 30 °C) and their interactions with 2 h exposures to these same temperature conditions were investigated. Lastly, the effects of temperature ramping rates on CTLs were investigated to better comprehend ecologically-relevant thermal limits to fruit fly activity.

Results: The range of temperatures inducing RCH were similar for both species (5-10 °C) but at 5 °C *C. capitata* developed RCH significantly faster than *C. rosa* and which also persisted longer (8 h vs. 0.5 h) than its congener. A simple population survival model, based on the estimated time-course of RCH showed that time to extinction is greater for *C. capitata* than for *C. rosa*, especially in habitats where temperatures frequently drop below 10°C. Acclimation experiments showed improvement in heat tolerance with high temperature acclimation and an increase in low temperature tolerance following acclimation to cooler conditions although in some cases, significant interaction effects were evident for CTLs between short- and long-term temperature treatments. Slower heating rates also resulted in higher CT_{max} while slower cooling rates elicited lower CT_{min}.

Conclusions: Variation in RCH responses may translate into significant variation in survival upon introduction to novel thermal habitats for *C. capitata*, particularly in cooler and more thermally variable geographic regions and may contribute to their ongoing invasion success relative to other, more geographically-constrained *Ceratitis* species. Acclimation results suggest that CTLs of adult fruit flies are moderated by temperature variation at both short and long timescales and may comprise both reversible and irreversible components

Keywords: Acclimation; critical thermal limits; fruit flies; invasive; rapid cold-hardening

FEMALE RECEPTIVITY MODULATION IN *ANASTREPHA FRATERCULUS*: THE ROLE OF SPERM AND MALE ACCESSORY GLANDS PRODUCTS.

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Background: Renewal of receptivity in females may reduce reproductive success of the first male. It is expected that males develop ways to manipulate female receptivity and avoid mixture of rival's sperm. In several fruit flies, at least two mechanisms were examined: sperm effect and male accessory glands products (AGPs) effect. Here we evaluated the role of sperm and AGPs in the renewal of *A. fraterculus* female receptivity and how this was affected by the diet the male was provided and male origin.

Methods: First, females were mated with males fed either sugar, low quality protein (3:1 sugar: protein ratio), high quality protein (same ratio) and the standard adult diet (sugar, protein, vitamins and corn hydrolyze). Wild and laboratory flies were evaluated. After the first copulation, different sets of females were used to determine female remating rate, refractory period, and sperm stored. Second, we evaluated the receptivity of females microinjected with AGPs in two set of trials. In the first, laboratory females were injected with AGPs of laboratory males fed either with sugar or the standard diet. In the second, laboratory and wild females were injected with AGPs of laboratory and wild males, all of them fed with standard diet. Female receptivity was evaluated 48 h after injection and the control were females injected only with saline. Differences in the frequency of remating females among treatments were compared with χ^2 -tests with Bonferroni's correction. Refractory period and amount of sperm stored were analysed with ANOVA coupled with Tuckey's test.

Results: Wild females mated with sugar males presented a higher remating rate and a shorter refractory period than those mated with standard diet males. Amount of sperm stored in females mated with sugar or low quality protein males was significantly lower than that stored in females that mated with standard diet males. Laboratory females also showed a lower refractory period when mated with sugar males compared to females mated with protein males irrespective of the protein quality. However, remating rate and amount of sperm stored was not affected by the diet. Additionally, females injected with AGPs of laboratory standard diet males had lower receptivity than females injected with AGPs of laboratory sugar males, which in turn were equal to control females. Females injected with AGPs from laboratory males had lower receptivity than females injected with AGPs from wild males (which were equal to the control), irrespective of female origin.

Conclusions: Male nutritional status and origin affects female renewal of the receptivity. In wild flies this seems to be related with the sperm stored in the female. However in laboratory flies, the sperm effect seems to be not the only responsible of modulating these changes. Male nutritional status and origin also affected female receptivity trough an AGPs effect. The later may be related to the crowded conditions under laboratory rearing where laboratory males may need to increase the effectiveness of their AGPs to ensure its paternity.

Keywords: polyandry, remating inhibition, sperm effect, accessory gland effect, South American fruit fly.

ABUNDANCE OF FRUIT FLY SPECIES AND THEIR PARASITOIDS COLLECTED ON COFFEE CROPS UNDER SHADED AND SUN CONDITIONS IN COLOMBIA.

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Background: This research is being conducted in order to identify *Anastrepha* species and their parasitoids related to two conditions of coffee systems: shaded and sun exposed coffee crops.

Methods: Twenty-four McPhail traps with protein hydrolysate as feeding attractant were placed randomly on five farms in four departments, Quindio (1), Caldas (2), Risaralda(1) and Valle del Cauca (1), located at the main coffee crop region in Colombia. Half the traps were disposed in shaded coffee crops, while the others were set under sun exposed conditions. The shaded trees species were as follow: avocado *Persea americana*, guamos *Inga* spp., guava *Psidium guajava*, and *Manga Mangifera indica*. Sun exposed did not have any other tree species at all. Fruit fly adults have been weekly collected and registered from the traps up to eight months. We performed frequency and constancy analysis with this information. Simultaneously, we collected 30 samples of coffee berries of a pound each, in order to calculate the percentage of parasitism and to register parasitoid species.

Results: Results showed total captures of 10,112 fruit fly individuals, where 5,512 corresponded to females and 4,600 to males of the *Anastrepha* genus (Tephritidae: Diptera). *Anastrepha fraterculus* was the most frequent species accounting 80% of the total captures, followed by *A. obliqua* with 9% frequency and *A. striata* with 7%. Other five species were identified with only 4% of the total captures: *A. distincta*, *A. grandis*, *A. mucronota*, *A. manihoti*, and *A. pallidipennis*. While in Valle del Cauca, Quindio and Risaralda the species *A. fraterculus* was the dominant species ranging from 97 to 99% frequency, *A. obliqua* and *A. striata* represented 14% of the total captures in Caldas and *A. fraterculus* 65%. In the whole experiment, 77% of the fruit fly individuals were captured under shaded conditions. The parasitism found from the 30 samples evaluated was 18.5%. So far, three genera of parasitoids were registered only in coffee crops under shaded conditions: *Doryctobracon* sp., *Microcasis* sp. and *Utetes* sp. (Braconidae: Hymenoptera). *Doryctobracon* sp. was the most frequent species.

Conclusions: *Anastrepha* was the only fruit fly genus collected at the main coffee growing area of Colombia. *A. fraterculus* was the most frequent species at both conditions shaded and sun exposed coffee crops.. Other seven species of fruit flies were identified living on coffee ecosystems. We found three parasitoids species of fruit flies under shaded coffee crop conditions which belonged to the Braconidae family.

Keywords Fruit flies, Coffee grown, *Anastrepha*, Braconidae.

FEMALE *ANASTREPHA SUSPENS*A (LOEW) RESPONSE TO THE VIBRATION COMPONENT OF MALE WING-FANNING SIGNALS.

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Background: *Anastrepha suspensa* is an important pest of fruit crops in the Caribbean region. Courtship and mating behaviors have been analyzed in many previous studies to develop control methods. During courtship, males group in leks on leaves of host trees, fan their wings, and release pheromone to attract females. The wing fanning vibrations are transferred through their legs to the leaves and nearby stems. We hypothesized that females detect these wing-fanning vibrations, and their behavioral responses may affect the likelihood of mating.

Methods: Wing-fanning vibrations of male *A. suspensa* on leaves of strawberry guava host branches were recorded with accelerometers. The vibrations were similar in spectral and temporal pattern to sounds detected simultaneously by microphones. A 2-min file was created from a representative, 11-s period of male calling with 1 min continuously repeating the 11-s call, followed by 1-min silence. The file was played back in an endless loop by a vibration exciter attached to a mesh cage containing 10 females. The behaviors of the females were observed remotely at the midpoint of each 1-min calling or silence period using a video camera during 20-min playbacks. Movement was defined as activities typical of avoidance or disturbance behavior, including spinning around, walking, wing-fanning, and flying. If the female was resting or grooming at the calling or silence midpoint, she was scored as quiet. The total numbers moving or quiet at each midpoint were summed to score each test. Two groups of females were tested to consider possible effects of prior exposure to males. One group (13 tests) was exposed to pheromone produced by a cage of calling males on the day previous to testing. The other group (12 tests) was never exposed to males or male pheromone prior to testing. All tests were conducted during times of day optimal for mating.

Results: Fewer females moved during the periods when calling vibrations were played than during periods of silence. For females who had never been exposed to male pheromone, a mean of 12.7 females per 20-min test were moving at the midpoint of the vibration period compared to 16.8 at the midpoint of the silent period ($t = -2.44$, $df = 12$, $P = 0.03$), i.e., 0.13 movements/fly/min of broadcast compared to 0.17 movements/fly/min of silence. When females were pre-exposed to males on the day before testing, a mean of 14.4 were moving at the midpoint of the vibration period compared to 19.25 at the midpoint of the silent period ($t = -2.84$, $df = 11$, $P = 0.02$) or 0.14 movements/fly/min of broadcast compared to 0.19 movements/fly/min of silence.

Conclusions: The results suggest that female *A. suspensa* can detect the wing-fanning vibrations from the male calling song on the host plant, just as they detect the sounds of the calls, and that the vibrations may reduce the likelihood that the female will move away from the male who approaches her to mate. The observed effects of vibrations are similar in many respects to the effects of wing-fanning sounds on females, and the two signals may have synergistic effects. Could disruption of male vibrations be a potential control method?

SPATIAL AND DIURNAL PATTERN OF PROTEIN FORAGING BY *BACTROCERA TRYONI* (FROGGATT) ON A HOST PLANT.

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Background: Queensland fruit fly, *Bactrocera tryoni*, is the major pest fruit fly in Australia. Protein bait sprays, where insecticides are mixed with spot applications of a protein based food lure, are one of the sustainable pre-harvest fruit fly management strategies used in Australia. Although protein bait sprays do manage fruit fly infestation in the field, there is little science underpinning this technique and so improving its efficacy is difficult. Lacking information includes where and when to apply protein bait in order to best target foraging *B. tryoni*. As part of new work in this area, we investigated the effect of height of protein on tree and host plant fruiting status on the spatial and temporal protein foraging patterns of *B. tryoni*.

Methods: The work was conducted in the field using nectarine and guava plants and wild *B. tryoni* at Redland Bay, Queensland, Australia. Spot sprays of protein bait were applied to the foliage of randomly selected fruiting and non-fruiting trees. Each tree received protein bait spot sprays on the lower and higher foliage at 0530hrs. The number, sex and species of flies that fed on each protein spot were recorded hourly from 0600hrs through to 1800hrs.

Results: For nectarines, there was a significant difference in the number of *B. tryoni* feeding on protein bait placed at different locations within the tree (ANOVA, $F = 8.898$, $p = 0.001$). More flies fed on protein placed on higher foliage relative to lower, irrespective of the fruiting status of the nectarine trees. A significant difference was also observed in the diurnal protein feeding pattern of *B. tryoni* (ANOVA, $F = 2.164$, $p = 0.024$), with more flies feeding at 1600hrs. Results for guava are still being collected and will be presented at the meeting.

Conclusions: We conclude that *B. tryoni* effectively forages for protein at heights higher than 1.3m from ground, indicating greater efficacy of protein bait when applied at foliage higher in the canopy. *Bactrocera tryoni* actively forages for protein throughout the day, with a highest feeding peak at 1600hrs. The lack of significant difference in the spatial protein foraging pattern between fruiting and non-fruiting nectarine trees may be a real result, or may have resulted from the fruiting tree being very close (within 1 – 2 metres) of the non-fruiting tree. This hypothesis is being tested in the guava trial.

Keywords: protein bait, resource use

CLASSICAL AND MOLECULAR STUDY OF GUT BACTERIAL COMMUNITY STRUCTURE IN ALIMENTARY TRACT OF *BACTROCERA CUCURBITAE* (COQUILLET).

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Background: *Bactrocera cucurbitae* (Diptera: Tephritidae) is recognized as an important insect pest species in many cucurbitaceous plants. The bacteria in fruit flies alimentary tract have been known in advantages in their biology of the insect hosts. The classical techniques of gut bacterial study in fruit fly were known with easy culturable gut bacteria but unculturable and not-yet culture bacteria could not be detected. Recently, molecular techniques can reveal real time of gut bacterial community structure in insect alimentary tract. In this research, we employed classical and molecular cloning techniques to study gut bacterial community structure in *B. cucurbitae* gathered from wild.

Methods: Both males and females of *B. cucurbitae* were hand-collected from their natural host plants in Nakhon Si Thammarat province, Thailand. Crop and midgut of the flies were dissected with aseptic techniques. The classical technique was the isolation of the gut bacteria on culture media and identified with API 20 E test kit. For the molecular technique, DNAs were extracted from their alimentary tracts. Genomic DNAs were amplified with universal primer of 16S rRNA gene for bacteria. PCR products were transfer to competent cell and selecting by cloning techniques. The clone containing interested gene were sequenced and analyzed on RDP II. The community diversity and community similarity were analyzed with Shannon-Weaver index, rarefaction analysis, Morisita's index, multidimensional scaling analysis (MDS) and phylogenetic tree analysis.

Results: Classical technique revealed only one bacterial family *Enterobacteriaceae* that belonged to *Gramma-proteobacteria* in this fruit fly species. In molecular study, five bacterial taxa, *Alpha-*, *Beta-*, *Gramma-proteobacteria*, *Actinobacteria* and *Firmicutes*, were found. The former three bacterial taxa were classified in phylum *Proteobacteria*. Interestingly, *Wolbachia* sp., known as bacterial arthropod pathogen and unculturable bacterium, was detected. Female midgut showed the highest values of gut bacterial community diversity. The gut bacterial community similarity competently distinguished the differences of their gut bacteria harbored between crop and midgut.

Conclusions: Molecular cloning techniques in this study have exposed appreciation of the ecology of gut bacterial community structures in *B. cucurbitae* alimentary canal. More bacterial species, including unculturable bacteria and also rare species were detected. *Grammaproteobacteria* was the predominant bacterial taxon in this fruit fly species.

Keywords: Gut bacterial community structure, Alimentary tract, Molecular cloning technique, *Bactrocera cucurbitae*, *Gramma-proteobacteria*

Session 2
Morphology & Taxonomy

SIMULTANEOUS DETECTION AND IDENTIFICATION OF TEN DIFFERENT SPECIES OF TEPHRITID FRUIT FLIES USING OLIGONUCLEOTIDE MICROARRAYS.

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Background: Tephritid fruit flies are the most destructive insect pests of horticultural crops worldwide. To prevent the invasion of exotic tephritids, many countries set strict regulation on the quarantine inspection for imported fruits and vegetables. A method for rapid identification of the species of intercepted pests, which are most at their larval or even egg stages, are required to enhance the efficiency and quality of quarantine inspection and service. Here, we report that we have developed a microarray to meet the demand for rapid identification of insect species.

Methods: Ten species of tephritid fruit flies, *i.e.*, *Bactrocera dorsalis*, *B. cucurbitae*, *B. tau*, *B. oleae*, *B. papayae*, *B. tryoni*, *B. carambolae*, *Ceratitis capitata*, *Anastrepha suspensa*, *Rhagoletis pomonella*, were selected for the experiment. The mitochondrial genes, including cytochrome oxidase I and II (COI and COII) and NADH dehydrogenase subunit 4 and 5 (ND4 and ND5), were targeted for species-specific probe screening. The genes were amplified by PCR using appropriate universal primers; the PCR products were cloned and sequenced; and the DNA sequences of each gene of all species were aligned for designing oligonucleotide probes. The probes were spotted on the microarray polymer substrate and immobilized by a UV cross-linker. The hybridization and colorimetric reactions between each target DNA and probe were carried out with DR. Chip DIYTM Kit (DR. Chip Biotech, Miao-Li, Taiwan).

Results: (1) a multiplex PCR with four pairs of primers, *i.e.*, COI, COII, ND4 and ND5, were developed, and it was able to simultaneously amplify all four target DNA fragments of all tested flies. (2) Multiplex probes of different oligonucleotide sequences targeting the four genes were obtained, and they were capable of discriminating one species unambiguously from others except between *B. dorsalis* and *B. papayae*.

Conclusions: We have developed an inexpensive microarray that enables to rapidly and simultaneously identify at least ten species of major tephritid pests within 5 hours.

Keywords: tephritid fruit fly, microarray, cytochrome oxidase, NADH dehydrogenase, plant quarantine

A HOLISTIC APPROACH TO DETERMINING SPECIES LIMITS BETWEEN MORPHOLOGICALLY SIMILAR PEST SPECIES OF THE *BACTROCERA DORSALIS* SPECIES COMPLEX: INITIAL RESULTS USING GEOMETRIC MORPHOMETRICS.

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Background Particular pest species of the *Bactrocera dorsalis* species-complex are taxonomically indistinguishable with high intra-specific morphological variation. The most problematic species are *B. dorsalis sensu stricto*, *B. papayae*, *B. carambolae*, and *B. philippinensis*. Doubt remains over the taxonomic validity and species limits of these taxa. We aim to test this hypothesis by examining different biological aspects of these species.

Methods The broad study incorporates multiple lines of evidence including cross-mating trials and comparing physiological characteristics among these species. This information will be coupled with genetic, morphological, and morphometric data. Work completed includes a preliminary geometric morphometric analysis of collection material to resolve fine scale differences among these taxa as currently described. For the geometric morphometric study, 15 landmarks were applied to wings of 245 collection specimens coming from three populations of each of the above mentioned species, plus one outgroup, *B. tryoni*. Generalized Procrustes analysis was then performed, from which canonical variates analyses (CVA) were undertaken to determine if defined species could be discriminated. Hypothetical ‘unknown’ samples were also included to assess if this technique could assign unknown individuals to species based on shape data.

Results The geometric morphometric study revealed that intraspecific wing size did not vary significantly between populations within species, but significant interspecific size comparisons did occur. Of the ingroup species, *B. papayae* had the smallest wings (average centroid size = 6.002 ± 0.061 s.e.), while *B. dorsalis* s.s. had the largest (6.349 ± 0.066). CVA clustered together populations within a single species, but discriminated between species. The single exception was an intraspecific difference between one population of *B. carambolae* and the two other conspecific populations. This statistically unusual population was subsequently deemed an original misidentification and likely represents an undescribed species. Individuals were correctly reassigned to their respective species on average 93.27% of the time; the assignment of 15 ‘unknowns’ was 80% successful.

Conclusions We conclude that geometric morphometric data is applicable to quantifying shape variation for these species. With the generation of further biological data, we anticipate our understanding of species limits for this group will significantly improve, as will our ability to develop diagnostic markers.

Keywords: geometric morphometrics; species limits; cryptic species

THE *ANASTREPHA FRATERCULUS SPECIES* COMPLEX: RECOGNITION OF THREE MORPHOTYPES FROM ANDEAN COUNTRIES USING THE MULTIVARIATE APPROACH.

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Background: One of the most important fruit fly species in continental America is the South American fruit fly *Anastrepha fraterculus* (Wiedemann), recorded from southern Texas (USA) through Mexico, Central America, and South America. This nominal species is currently recognized as a cryptic species complex, based on karyotypic differences, isozymic analyses, mitochondrial DNA sequences, or host use patterns, among others. Despite all this evidence, to date there are no taxonomic elements to ascertain how many species there are in this complex?, or how they can be distinguished from each other?. A recent morphometric analysis from several Mexican populations of the nominal *A. fraterculus* showed that they can be distinguished from other samples belonging to South America. In this study we assessed the morphometric variability using the same approach in ten natural populations collected from four South American countries.

Methods: We analyzed 10 population samples from Colombia (2), Venezuela (5), Ecuador (1) and Perú (2) using 15 specimens by sample. On the basis of structures as the aculeus, mesonotum and wing, 19 morphometric traits were assessed for each specimen. Discriminant function analysis (DFA) were applied to the full data set, grouping the specimens by locality of origin. The functions were tested by canonical correlation analysis to assess the significance of the discriminatory power of the model and variables involved in the segregation of the groups.

Results: Our results showed that there are clear differences between populations, even though all of them become from the biogeographical sub-region of Northwestern South America. Samples from the highlands of Colombia and Venezuela were very similar to each; a second group was differentiated from a single population of the Venezuelan lowlands; while samples from the lowlands of Peru and Ecuador were grouped separately.

Conclusions: In the Andean countries the *AF* complex would be represented by three natural groups here called morphotypes: the Venezuelan morphotype, which occurs at lowlands from that country; the Andean morphotype assembled by highland populations from Venezuela and Colombia; and the Peruvian morphotype recognized by populations that occur along the Pacific coastal slopes from Peru and Ecuador.

Keywords: South American Fruit Fly, cryptic species, morphometry, morphotypes

DIVERGENCE OF FRUIT FLIES IN THREE SPECIES GROUPS OF THE GENUS *RHAGOLETIS* IN MÉXICO.

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Background: Fruit flies in the genus *Rhagoletis* spearheaded the long and heated debate about the possibility and later the frequency of occurrence of sympatric speciation via host shifting for more than forty years. Once sympatric speciation was accepted as a common route to divergence in phytophagous insects, careful study of Mexican populations of the apple maggot fly *Rhagoletis pomonella* Walsh revealed that differentiation in the *pomonella* group had been in fact possible due to a combination of periods of allopatric divergence, gene flow and introgression and recent sympatric speciation via host shifting events.

Methods: Such findings led us to undertake detailed philogeographic studies of the genus in México. We first studied the distribution, divergence and evolution of reproductive isolation among hawthorn infesting populations of *Rhagoletis* in Mexico and the U.S. We are now conducting similar studies on black cherry infesting populations in the *cingulata* species group, and walnut infesting populations in the *suavis* species group.

Results: Despite the fact that flies in the three species groups shared a similar climatic history of range contraction and expansion, divergence in the three groups appears to be quite different, with flies in the *pomonella* group exhibiting what is now known as speciation mode plurality, flies in the *suavis* group undergoing rapid morphological variation without reproductive isolation and introgression between some species, and flies in the *cingulata* group exhibiting classic vicariant speciation.

Conclusions: We discuss hypotheses on the outcome of these studies.

Keywords: *Rhagoletis*, philogeography, divergence, speciation

Session 3
Genetics & Evolution

NEW TOOLS FOR THE DEVELOPMENT AND STABILIZATION OF TRANSGENIC LINES OF *CERATITIS CAPITATA* (WIEDEMANN).

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Background. The Mediterranean fruit fly (medfly) (Wiedemann; Diptera: Tephritidae), *Ceratitis capitata*, for which a successful SIT package has been developed and validated, was the first non-drosophilid insect to be transformed, opening the way for the genetic transformation of many other pest insects that are targets of SIT programmes. The efficacy and cost effectiveness of the SIT both at the mass-rearing, release and monitoring stages may be improved by using different medfly strains currently available, which provide i) genetic marking for the identification of transformed insects; ii) male-specific fluorescent sorting; iii) sexing for male-only strains; iv) reproductive sterility through embryonic lethality.

Methods. For the efficient application of SIT programmes, effective monitoring to assess the number and mating success of the released flies is essential. With this aim, we developed a powerful sperm-specific marking systems based on the spermatogenesis-specific *C. capitata* β 2-tubulin (Cc β 2t) promoter. Molecular characterization and preliminary laboratory competitiveness assays identified a target line which was then modified by the site-specific integration system from phage *phiC31*. Donor plasmids containing an *attB* site, with additional markers, and transposon ends were integrated into *attP* sites randomly integrated within transposon-based vector by *phiC31* integrase-mediated recombination. Finally, transposase-encoding ‘jumpstarter’ strains were created and mated to transgenic strains to produce post-integrational excision of transposon ends.

Results. Several lines were generated in which the reporter genes tGFP or DsRedEx are expressed in a stable, persistent and sex-specific manner during spermatogenesis. Fluorescent sperm can be isolated from testes or spermathecae. The marking does not result in fitness costs in preliminary laboratory assays. Particularly, males of one sperm-marked line showed no significant reduction in their overall fitness, transmitting their genes to the next generation in a competitive way. Site-specific integration experiments performed on this line resulted in the post-integrational excision of a transposon end, which left stably integrated transgene insertions that could not be further remobilized.

Conclusions. The development of a sperm marking system in the medfly and the subsequent stabilization of the transgenic lines represent a powerful tool since i) the use of such harmless transgenic markers are an ideal for the transfer of insect transgenesis technology from the laboratory to field applications; ii) effective and easily recognizable sperm marking will make novel studies possible on medfly reproductive biology which will in turn help to further improve environmentally safe, biological pest management approaches; iii) the newly developed integration and stabilization system will allow the combination of several transgene-encoded advantageous traits at evaluated genomic positions to generate optimized strains for pest control that minimize environmental concerns.

Keywords: sperm marking, insect pest management, phiC31 integrase, transgene stability.

GENETIC ENGINEERING OF THE OLIVE FRUIT FLY, *BACTROCERA OLEAE*, FOR USE IN THE STERILE INSECT TECHNIQUE (SIT).

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Background: The olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae), is the most destructive pest of olive fruit, causing considerable crop damage in the Mediterranean region and in California. Despite significant spending on insecticides the olive fly causes a reduction in crop yield and quality. Furthermore, some of the insecticides used are being phased out and olive fly is developing resistance to insecticides (even to relatively new insecticides such as Spinosad). The sterile insect technique (SIT) is a species-specific and environmentally non-polluting method of pest control that involves the mass release of sterile insects that has been very effective against other fruit flies. Previous SIT attempts using irradiated mixed-sex insects achieved only limited success at suppressing populations of *B. oleae*. The released sterile males mated with the released sterile females, instead of dispersing and seeking the wild-type females. It was believed at the time that sterile flies preferred to mate earlier in the day, leading to partial reproductive isolation from with wild population. A genetic sexing system to allow male only release is therefore seen as essential for olive fly SIT; while also providing the several benefits provided by current Medfly genetic sexing strains

Method: We used genetic transformation methods to develop engineered strains of olive fly ('RIDL strains') and used modifications of standard Medfly protocols to test strain performance.

Results: Oxitec has developed genetic sexing strains of olive fly based on a conditional positive feedback design already successfully applied in two other tephritid species; the Mediterranean fruit fly, *Ceratitis capitata*, and the Mexican fruit fly, *Anastrepha ludens*. The strains are 100% effective in separating sexes and also incorporate a fluorescent marker allowing easy monitoring in the field. Furthermore, since females die without a dietary supplement (tetracycline) that is not available in the wild, significant bio-containment is also inherent in this design of sexing strain. Oxitec is currently conducting laboratory and greenhouse mating performance tests with these sexing strains to further optimize them for SIT.

Conclusions: Our current RIDL strains of olive fly appear to give excellent sex-separation ('genetic sexing') without obvious negative effects on other performance parameters; our latest data on performance will be presented. The development of these strains greatly improves the prospects for the use of SIT to control field populations of olive fly.

Keywords: olive fly (*Bactrocera oleae*), RIDL[®], SIT, genetic sexing

GENOMIC SEQUENCING OF THE MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA*.

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Background: A joint project between the Baylor College of Medicine Human Genome Sequencing Center, the U. S. Department of Agriculture, and the University of Pavia was initiated in 2008 to sequence the whole genome of the Mediterranean fruit fly, *Ceratitidis capitata*.

Methods: The source material for the project was embryonic genomic DNA extracted from the medfly Ispra strain maintained as an inbred colony for more than 20 years at the University of Pavia, though this is considered to be polymorphic. Paired-end libraries were created for the 454 Titanium sequencing platform.

Results & conclusions: Pyrosequencing at 454 Titanium platform of paired-end libraries has produced 5,179 million bases of genomic DNA sequence after 13 fragment runs. This represents approximately 10x genomic sequence coverage with an average read length of 348 bp. An initial assembly of the fragment data using 454 Newbler v.2.3 (pre-release-9/14/2009) software produced 215,255 sequence contigs longer than 500 bp. The average contig size was 1,852 bp and the N50 was 2,709 bp, with the largest contig size of 70,001 bp. The current contig size data is sub-optimal, and is likely due to high repetitive DNA content and sequence polymorphism in the sample, negatively affecting the assembly. To improve the assembly product, new 3 kb insert paired-end libraries for Illumina sequencing have been created to order and orient the available contigs within the assembly, as well as to add data using a different sequencing technology. Sex-specific and embryonic RNAseq transcriptome sequencing is also anticipated. Upon completion of Illumina sequencing (expected in the summer/autumn of 2010) additional assemblies will be performed, and based on these results an interim assembly product will be released to public databases.

Keywords: Whole genome sequencing, Medfly, 454 Titanium sequencing, Illumina sequencing

APPLICATIONS OF GENOME SEQUENCING TO IDENTIFICATION, DETECTION, AND CONTROL OF *BACTROCERA DORSALIS* (HENDEL) AND OTHER TEPHRITID PESTS.

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Background: Recent advances in DNA sequencing technologies have increased the feasibility of performing large scale sequencing experiments in non-model organisms. Next-generation sequencing (NGS) technologies have allowed for whole genome shotgun (WGS) sequencing and assembly experiments to be performed in a matter of months for thousands of dollars compared to the years and millions that it previously took using traditional methods. The *Bactrocera dorsalis* genome project is one of the first projects to utilize only NGS technologies to produce a draft genome assembly of a non-model organism.

Methods: The sequencing of the oriental fruit fly genome was performed by using a combination of shotgun and paired-end (8 and 20 kb) sequencing with 454 pyrosequencing utilizing Titanium based chemistry. Approximately 8 Gb of sequence was obtained. A draft assembly of the genome, utilizing *de novo* assembly strategies was created using both the Newbler and the Celera assemblers. From this draft assembly, gene identification and annotation is currently taking place, utilizing computational pipelines that identify coding regions, compare the sequence to previously identified genes, and assign function annotation based on gene ontology. This draft database will likely contain the majority of the genes present in the oriental fruit fly genome, and can then serve as a database for functional genomics and proteomics as well as be used as a basis for developing molecular tools for use in other research projects.

Results: Pyrosequencing on a 454 sequencer using Titanium chemistry was able to produce a de-novo assembly at approximately 15x coverage. A draft genome of approximately 430 Mb in size was created. Currently, annotation and analysis of this dataset is ongoing. The utility of this dataset to the management of this and other Tephritid pests is broad, ranging from development of genetic markers, such as SNPs and microsatellites that can be utilized for molecular diagnostics, to functional genomics tools that can be applied to physiological questions, targeting insect resistance, olfaction, digestion, or other areas.

Conclusions: The integration of a genomic perspective into other research areas opens the door for many new approaches to asking fundamental questions in invasive species research.

Keywords: Oriental Fruit Fly, genome sequencing, functional genomics, genetic markers

HOST PLANT TOXICITY, STENOPHAGY AND EVOLUTIONARY RADIATION IN THE GENUS *CERATITIS* (DIPTERA, TEPHRITIDAE).

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Background: It is widely acknowledged that the evolution of insect-host relationships has been largely influenced by the chemistry of insect host plants. However, whether and to what extent secondary plant metabolites triggered the evolution of host plant specialization in phytophagous insects is still much debated. We here describe the phylogeny of host plant specialization within the genus *Ceratitis* and unravel the link with host plant toxicity as a possible mechanism that underlies the evolutionary radiation of stenophagous clades within this genus.

Methods: Using molecular data from three protein encoding genes and 49 species (98 specimens), we reconstructed the phylogeny of the genus *Ceratitis* and investigated the evolution of host plant specialization along the different recognized clades. We used HPLC analysis to compare the concentration of two toxic secondary metabolites (alkaloids) in host plants of stenophagous and polyphagous species. In an experimental set-up, we tested if, and to what extent, development and fitness of a polyphagous fruit fly is adversely affected by host plant toxicity by comparing rates of development, survival and reproduction on four media that differ in alkaloid concentration.

Results: Bayesian tree reconstructions supported previously proposed monophyletic lineages. Reconstruction of ancestral character states for host plant relationships suggested that stenophagy evolved repeatedly and independently within the genus *Ceratitis*. HPLC analysis proved an overall significantly higher concentration of toxic alkaloids in fruits of the stenophagous host plants, even though their concentration gradually decreased during ripening process. Despite reduced pupal and adult sizes, polyphagous larvae developing under low alkaloid concentrations successfully developed to the adult stage, probably as a result of accelerated pupation and ensuing restricted exposure to the toxic environment. High alkaloid concentrations, however, impaired their development process and prevented subsequent reproduction.

Conclusions: The proposed phylogeny shows a number of biological clades including stenophagous species which share host genera with genus-specific toxic secondary metabolites. The adverse effects of host plant toxicity on larval development in polyphagous fruit flies presented here indicate that these high concentrations pose a significant constraint on host use in polyphagous *Ceratitis* species. We conclude that the observed phylogenetic patterns for monophyletic stenophagous clusters are the result of an evolutionary process of ecological specialization to toxic hosts.

Keywords: *Ceratitis*, phylogeny, host-plant toxicity, stenophagy, evolutionary radiation

MACROGEOGRAPHIC POPULATION STRUCTURING IN THE COSMOPOLITAN AGRICULTURAL PEST *BACTROCERA CUCURBITAE* (DIPTERA: TEPHRITIDAE).

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Background: The melon fly *Bactrocera cucurbitae* (Coquillett) is a major, highly polyphagous, agricultural pest abundant throughout the African continent, the islands of Indian Ocean, Asia, New Guinea, the Mariana Islands and Hawaii. This species attacks more than 125 plants including commercial crops such as pumpkin, cantaloupe, watermelon, squash, gourd, cucumber, tomato, eggplant and bean as well as soft fruits such as mango, orange, papaya and peach. Although the economic importance of *B. cucurbitae* is well documented, its large-scale (*i.e.* inter-regional) patterns of genetic structuring are poorly known. Hence, the current quarantine methods and management plans still rely on general assumptions concerning its intraspecific variation. The objectives of this work were to 1) characterize the large-scale population structure of *Bactrocera cucurbitae*, 2) identify its geographic origin and 3) infer the dynamics of its range expansion.

Methods: Individuals of *B. cucurbitae* were collected from 25 worldwide-distributed localities (n=570) and genotyped at 13 microsatellite loci specifically developed for this species. Genetic discontinuities among geographical regions and levels of population admixture were quantified through Bayesian clustering procedures

Results: Five main groups of populations were identified. These corresponded to populations from 1) the African continent, 2) La Réunion, 3) Central Asia, 4) East Asia and 5) Hawaii. The proportions of inter-regional assignments and the higher values of genetic diversity in populations from Pakistan, India and Bangladesh suggest that *B. cucurbitae* originated in Central Asia and expanded its range to East Asia and Hawaii on one hand and to Africa and the islands of the Indian Ocean on the other. A number of outliers (10-19 specimens according to different clustering algorithms) show high levels of admixture ($Q > 0.70$) with populations from different regions and reveal complex patterns of inter-regional gene flow.

Conclusions: Anthropogenic transport is the most plausible promoter of the large-scale dispersal of *B. cucurbitae*. The recent African invasions of *B. cucurbitae* were mainly due to the expansion of local populations while, hitherto, the introduction of individuals from geographically distant regions did not have a relevant role. The dynamics of the recent invasions, as well as the possible corridors to gene flow created by contemporary anthropogenic activities could provide a useful background to better evaluate invasion risks and establish priorities for the management of this cosmopolitan agricultural pest.

Keywords: Tephritidae, agricultural pests, biological invasions, population structure, microsatellites.

GENETICAL CHANGES IN *BACTROCERA TRYONI* DURING LABORATORY ADAPTATION, AND THE USE OF FOUR-WAY HYBRIDS TO REDUCE INBREEDING EFFECTS

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Background: Domestication of wild strains for use in factories appears to be a problematic process in Queensland fruit fly (*Bactrocera tryoni*), as in most tephritids. Early generations, in particular, are subject to large fluctuations apparently due to fertility bottlenecks. These are expected to lead to genetic changes due to the twin factors of inbreeding and selection, with associated detrimental effects on the performance of released SIT flies. The purpose of the present experiment is to use microsatellite markers to investigate the relative importance of these two factors in the process of introducing wild strains into the laboratory.

Methods: Eight populations were set up using flies collected from different areas of the Sydney region. These populations were sampled over ten generations. All samples were typed for nine of the most variable of the established Q-fly microsatellites. For six of the eight lines, 48 flies were sampled at generations 0, 1, 2, 3, 4, 6 and 10. For the remaining two lines, 48 flies were sampled at generations 0, 1 and 10. Data on the distribution of individual age-specific female fecundity were also collected.

Results: The rate of inbreeding was shown to be highly repeatable. The observed inbreeding process was modelled by stochastic simulation of the random genetic drift occurring in the replicates line, taking into account the finite population size and distribution of family sizes. The model successfully predicted the rate of decline of heterozygosities in the replicate lines, showing that the inbreeding in new Q-fly lines is unavoidable, even in the absence of “hard” selection for increased early-age egg production. There was, however, also some evidence for selection as shown by repeatability of changes in two lines. Comparisons between factory flies and hybrids showed that it is possible to retain the high fertility of factory flies while improving performance in dispersal activity and stress resistance.

Conclusions: Both selection and inbreeding are expected to have undesirable consequences in terms of the performance of SIT releases. This experiment indicates that inbreeding per se is a likely factor in the early generations of laboratory culture. It follows therefore, that measures to reduce the effects of inbreeding during the setting up of factory strains are of value. A protocol using four-way hybrids is outlined.

Keywords: microsatellites, Q-fly, inbreeding

A GENETICS COMPARATIVE WORK OF TWO DIFFERENT TEPHRITIDAE SPECIES, *CERATITIS CAPITATA* (WIEDEMANN) AND *BACTROCERA OLEAE* (GMELIN): RAPD DATA.

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Background: The olive fruit fly, *Bactrocera oleae*, is a major pest of olive crops and its expansion is exclusively restricted to the cultivation zone of the olive tree. The medfly, *Ceratitis capitata*, however, is a highly polyphagous species that infest more than two hundred different vegetal species. Both constitute important pests in the Mediterranean area being responsible of important crops damages and economic losses. We report here a comparative work on the genetics structure of their populations based on RAPD molecular markers information.

Methods: Flies from different geographical areas, mostly Mediterranean, were collected by harvesting infested fruit and allowing the larvae to pupate in the laboratory. Individuals from 20 different locations for *C. capitata* and 21 for *B. oleae*, were analyzed on the basis of Random Amplified Polymorphic DNA through the Polymerase Chain Reaction (RAPD-PCR). Genomic DNA was extracted from individual flies according to Reyes, Ochando *et al.* (1997), and amplified with six or seven different primers (Sets A and C, Operon Technologies). DNA amplifications were performed under the conditions reported by Williams *et al.* (1990), with slight modifications. Each amplification reaction was performed at least twice: the results were consistently reproducible. The amplification products were separated according to their molecular size by electrophoresis in 2% agarose gels with TAE buffer.

Results: The observed polymorphism was high in both species, even higher in *B. oleae* (0.49 for *Ceratitis* and 0.66 for *Bactrocera* species). With respect to the distribution of the genetic variability, both species showed the same pattern. In both species the within population variation (between 76 and 90 %, AMOVA analysis) was significantly higher than the between populations variation. Genetics distances were low and similar for all North Mediterranean samples in both species without clear phylogenetics relationships. However the rest of the populations (Tunisia, Israel and USA for *B. oleae*, and Kenya, SouthAfrica, Tunisia and Israel for *C. capitata*) were clearly different.

Conclusions: However the different ecological life of the two analyzed species, *C. capitata* and *B. oleae*, their genetic variabilities are roughly of the same level from the quantitative point of view and show similar characteristics from the qualitative point of view. Thus, the ecological and feed parameters can not explain those coincidences. Other aspects such as effective population numbers, gene flow and elapsed time from the colonization processes, must be taking into account for population structure in these pests.

Keywords: *C. capitata*, *B. oleae*, RAPD-PCR, polymorphism, genetic distances.

Session 4
Risk Assessment, Quarantine &
Post-harvest Treatments

QUARANTINE SECURITY FOR COMMODITIES: DETERMINING THE NON-HOST STATUS OF FRUITS REGULATED FOR TEPHRITID FRUIT FLIES

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Background: Assessing the risk of fruit fly pests associated with the movement of various economically important commodities is paramount in facilitating global trade. Determining the fruit fly host status of a particular commodity is imperative in developing risk mitigation measures and establishing the intensity of quarantine treatments. Host suitability is a critical quarantine security parameter of several international and regional standards. In particular, we aim to review RSPM 30: “Guidelines for the Determination and Designation of Host Status of a Commodity for Fruit Flies (Diptera: Tephritidae).” Here we report on the parameters defined in RSPM 30 that we used in determining the host status of ‘Hass’ avocados in Peru to infestation by South American fruit fly, *Anastrepha fraterculus* (Diptera: Tephritidae).

Methods: Experiments were conducted, following the RSPM 30 guidelines, to determine the host suitability of ‘Hass’ avocados to *A. fraterculus* under natural field conditions in five orchards located in Lima, Ancash and Piura. Both wild and laboratory-reared adults were used. The adult population density in test orchards was estimated by trapping and collecting natural host fruits. During the entire harvest season, commercial grade mature green ‘Hass’ fruits were collected biweekly from trees in each test orchard, and the infestation level of *A. fraterculus* determined by peeling (removing skin and cutting pulp) and holding fruits in rearing containers. *A. fraterculus* infestation in fruits on the ground and culled fruits from packing houses was also determined. No-choice and choice oviposition tests using intact and punctured fruits were conducted in cages measuring 4-m high and 4-m diameter which covered an entire tree. No-choice forced oviposition tests were also conducted using sleeves measuring 1.5-m length and 1-m diameter that enclosed an entire branch. For oviposition tests, mangoes were used as control fruits.

Results: Successful insertion of eggs by *A. fraterculus* into the skin (exocarp) and pulp (mesocarp) of mature green ‘Hass’ avocados was never observed in both choice and no-choice forced oviposition tests. All fruit samples collected from the field and packinghouses had no *A. fraterculus* infestation. The population density of *A. fraterculus* inside and outside the orchards was low.

Conclusions: Based on the studies conducted following the guidelines of RSPM 30, mature green, commercial grade ‘Hass’ avocados appear to be conditional non-hosts of *A. fraterculus*, suggesting that the current mitigation options for *A. fraterculus* need to be evaluated based on the present results.

Keywords: quarantine security, host suitability, systems approach; fruit flies; ‘Hass’ avocado

THE POTENTIAL GLOBAL DISTRIBUTION OF NATAL FRUIT FLY, *CERATITIS ROSA* (KARSCH) (DIPTERA: TEPHRITIDAE).

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Background: *Ceratitis rosa* is an important pest of various commercially grown fruit crops in South Africa. Its present distribution is restricted to Africa, Mauritius and Reunion. Phytosanitary restrictions are imposed on international fruit trade to mitigate against the risk of introducing this pest into importing countries. Knowledge of its potential to invade other parts of the world makes it possible to evaluate the relevance of such measures. The potential future global distribution of *C. rosa* has previously been modelled using correlative species distribution modelling techniques. However, these models were built on presence data and did not incorporate information on the species' relative abundance and seasonal phenology. The wide range of climatic conditions in South Africa, and related differences in the species' abundance, makes the region well suited to obtain information on relative abundance and seasonal phenology of the species in relation to climate. The objective of the study was to model the potential global distribution of *C. rosa* based on its distribution, relative abundance and seasonal phenology in South Africa.

Methods: Yellow bucket traps, baited with Biolure® Fruit Fly, were used to trap *C. rosa* in different climatic regions of South Africa. Traps were placed in host plants and serviced monthly for a two-year period. A CLIMEX niche model of the potential global distribution of *C. rosa* was fitted based on the collected trapping data and other distribution records from South Africa. Distribution records for elsewhere in Africa were reserved for independent model validation. CLIMEX is a generic dynamic simulation model that combines inferential and deductive modelling approaches. It can be used to infer what climatic conditions a species can tolerate based on where it lives or its phenology.

Results: The CLIMEX model output conformed well to the data observed from trapping in South Africa, not only in terms of the species' distribution, but also in terms of the relative abundance and seasonal phenology. The projected distribution also accords well with the pattern of presence records of the species elsewhere in Africa. The model predicts a large part of South America, Central America, Mexico and southern USA to be climatically suitable under recent climate conditions. In Europe, climatically suitable habitat is restricted to the coastal regions of the Mediterranean. In Asia, suitable climate is restricted mostly to the southern and south eastern countries, while in Australia it is mostly the wetter South and East.

Conclusions: The independent cross-validation provided by relative abundance and seasonal phenology data, together with relevant species specific biological information enhanced the modelling of the species' potential global distribution, thereby producing a more reliable model.

Keywords: Biosecurity, CLIMEX, distribution, relative abundance, seasonal phenology

ASSESSMENT OF THE RELIABILITY OF EXISTING PHYTOSANITARY MEASURES AGAINST THE INTRODUCTION OF NON-EUROPEAN TEPHRITIDAE INTO THE EUROPEAN COMMUNITY.

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Background: The *Tephritidae* family comprises 500 genera, including the *Anastrepha*, *Bactrocera*, *Ceratitis* and *Dacus*, and *Rhagoletis* genus, worldwide notorious for their destructive impact on agriculture. Except for *Rhagoletis* spp., which are found in temperate areas of Europe, few species of the other genera occur within the European Community (EC). As it includes transboundary pests of global concern, a key issue for pest risk analysts is to assess the reliability of the existing phytosanitary measures. We summarize here the current EC set of measures against non-European *Tephritidae*, identify their efficacy and compare them with those applied in third countries.

Methods: The EC Plant Health Directive has been conscientiously revised in order to identify all references to *Tephritidae* and plant products with which the fruit flies have a possibility of being associated in a suitable life stage. We have checked the ability of fruit flies to survive the standard shipping methods by consulting the European interception database (Europhyt). Finally we have selected from the relevant pathways of introduction those which appear to pose a higher risk for the EC.

Results: Two major pathways have been identified: (i) fresh fruits of host plant species and (ii) plants for planting (except seeds) accompanied by contaminated, attached growing media from countries where non-European *Tephritidae* occur. Frequently, only plants and plant products which are accompanied by a Phytosanitary Certificate are subject to a plant health inspection in the ports of entry. This common practice hinders the efficacy of the protective measures laid on the Directive and lead to visual inspection as the only requirement against fruit flies for most fruits (citrus are an exception). Regarding soil and growing medium attached, the recurring detection of harmful nematodes in these consignments shows the lack of implementation in practice of the requirements imposed to the EC trading partners.

Conclusions: Visual inspection, as a single measure, is not enough to guarantee that fruits and plants with attached growing media are free from tephritids. This statement is supported by the regulation in force in third countries (e.g. USA, China, Australia or Japan) that do not rely solely on visual inspection of consignments. The EC, as major importer of plant products, is exposed to the entry of non-European *Tephritidae* and should impose stricter quarantine measures to prevent their spread within its territory.

Keywords: Pest Risk Analysis, phytosanitary measures, reliability, pathway, introduction.

DESIGNING PRACTICAL LABORATORY PROCEDURES FOR DETERMINING HOST STATUS OF COMMODITIES TO FRUIT FLIES USING VARIOUS *ANASTREPHA* SPECIES AS EXAMPLES.

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Background: A critical component of assessing pest introductions and requirement for quarantines in fresh fruits/vegetables is the host status that determines risk of pest transport and introduction. Here we present a summary of laboratory procedures that address host evaluation and illustrate the procedure with summaries of laboratory experiments with the Mexican fruit fly, *Anastrepha ludens* Loew, the West Indian fruit fly *A. obliqua* (Macquart), and the Sapote fly *A. serpentina* (Weidemann) in tests with various Citrus species and cultivars. Based on earlier tests, we classified host status as either natural hosts or conditional hosts, and fruit fly species were classified as natural pests or conditional pests.

Methods: Experiments tested survival and development of eggs and larvae of the fruit flies to compare tissue specific survival of the developmental stages of these species in natural and conditional citrus hosts and determine the tissue specific survival rates related to the maturity of the fruit. Experiments were performed by exposing fruit in laboratory cages (30 cm cubic/ 8 fruit per cage, 8 mature females/fruit) using fruit wrapped to permit oviposition in a marked area for 24 h. Tests comparing fruit fly species were run in separate cages concurrently. Fruit were unwrapped, held in containers for maturation, then samples were dissected after 3 d, and weekly for 4 weeks to determine tissue and time specific survival of 4 (egg, and 1st-3rd instar) stages in flavedo, albedo, and pulp.

Results: In all tests most mortality occurred during the first week after oviposition for eggs and first stage larvae in the albedo. The Mexican and sapote fruit flies oviposited into the albedo but West Indian fruit fly eggs were found mostly in the flavedo among the oil glands. Results showed large differences among the species for factors such as fruit maturity and senescence affecting mortality in the albedo but mortality was very low in larvae reaching the pulp for any of the species.

Conclusions: The methods described here allowed identification of factors that affect host status for the 3 *Anastrepha* species of high economic importance in North America. The method could be tested under laboratory conditions during a single season. The Mexican fruit fly which is an economic pest on most citrus (but not Eureka lemons) was most sensitive to fruit maturity. Fly trapping triggers for quarantines in areas using a systems approach to meet requirements could be adjusted based on these factors for conditional hosts.

Keywords: host status, quarantine

Session 5
SIT Principles & Application

IMPROVING MASS REARING TECHNOLOGY FOR THE MEDFLY TEMPERATURE SENSITIVE LETHAL STRAIN (TSL): GEL LARVAL DIET DEVELOPMENT AND VALIDATION.

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Background: Mass rearing technology is a crucial element to establish a functional Sterile Insect Technique (SIT) program for any species. Insect rearing represents approximately 50% of the SIT cost. A Gel larval diet and its rearing system for Medfly (*Ceratitidis capitata*) production were developed at San Miguel Petapa Facility in Guatemala. Benefits of a Gel diet technology include a reduction in waste diet; elimination of the bulking agent and reductions in diet ingredient storage needs and labor.

Materials and Methods: Medfly eggs for both male only and colony productions lines were produced at the El Pino Facility, Guatemala. Eggs batch samples (250 ml for colony and 700 ml for male only) were divided into equal parts. Half of the samples were sent to the Petapa Facility where the eggs were transferred onto the Gel diet. The diet is composed of Torula yeast, sugar, antifungal agents (sodium benzoate), hydrochloric acid, tap water and organic gel agent. Five replicas were conducted once per week during 5 consecutive weeks. Each experiment included approximately 60 trays for colony and 60 trays for male only production. The trays were loaded with two kilos of diet then male only trays were infested with 5.2 ml of eggs while the trays dedicated to the colony were infested with 1.8 ml of eggs. At El Pino the control eggs were seeded following normal rearing procedures. Trays were loaded with 5 kg of corn cob diet; male only trays were infested with 6.4 ml of eggs while colony trays were infested with 3.2 ml of egg. In both Facilities maturing larva were collected and measured every day to determine larva production quality. During the pupal development stage samples were separated and the quality assessed using standard QC tests.

Results: Larval rearing of Medfly on this gel diet resulted in 30% increase in pupal production while increases in the colony production line resulted in 47% more female pupal production as compared to the control diet. Pupal weight, adult emergence, adult fliers, egg hatch and egg production are basically equal to insects reared on a conventional corncob diet. This first mass rearing assessment has shown a significant saving (23%) on the total cost of diet to produce the same quantity of insects. While it is now expected that the Gel diet can save some diet related costs during the rearing process other operational components such as energy consumption, labor, space, etc. need to be considered in the overall calculation of cost savings.

Conclusion: The utilization of Gel diet technology may allow for considerable cost savings. However more research will be necessary in the future to design the best equipment and facility configuration for this new technology. The diet formulation may need to be revised so that more savings could be achieved without compromising the quality of the insects.

Keywords: Medfly, SIT, Medfly larval diet, Mass rearing

METHODOLOGY AND SAMPLE SIZE TO ESTIMATE RECOVERED FLIES AND ABSOLUTE FLYER FLIES POST-CHILLING PROCEDURE OF STERILE MEDFLY, *CERATITIS CAPITATA* (WIEDEMANN).

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Background: The efficacy of the Sterile Insect Technique is based on the quantity and quality of the adults released, which could be determined by the percent of post-chilling recovered flies (quantity) and absolute flyer flies (quality) after shipment, packaging, emergence, feeding, chilling and collection processes. In this work we described the adapted methodology for the daily estimation of the two parameters mentioned above for sterile male flies of *Ceratitits capitata* in Tapachula, México.

Methods: The evaluation was carried on using emergence tower type “Guatemala” conformed by 25 double-screen tap trays. In 20 trays we collocate ~25, 000 pupae that were previously weighted. The trays remained in the emergence room at 24 °C and 60% RH, with food in form of fine flour (90% sugar, 10% vegetable proteins) and water. On the fifth day, the trays were moved into a cold room to slow down adults at 0 – 3 °C for 30 minutes.

To determine the recovered flies and absolute flyers, standard procedures using total content of each screen-tray were compared with the new proposed method, which consisted in a sample of 30 ml of chilled flies from each tray. We used the proportion 9:1 between the weight of adult fly and its puparia as a constant. Each sample was weighted and placed in a plastic container of 33 x 20.5 x 10.6 cm talc impregnated, which were collocated in open space at environmental temperature to stimulate the flies flying and to get the residue of non-flying flies. The percent of recovered flies was estimated from the difference of the initial average weight of pupa and the final weight or residue inside the containers. To calculate the sample size we used the formula $nE = (S / Ex)^2$ (FAO/IAEA/USDA 2003).

Results: The average percent and SD of recovered flies was 80% ± 0.62, while absolute flyer flies was 91 % ± 1.21. These results were compared with those obtained from standard procedures, 88% and 91%, respectively. The average weight in the sample of absolute flyers was 7.24 ± 0.36; using the formula with an accuracy level of 0.1 (90% reliability), the estimated number of samples was $(0.36/(0.0135*7.24))^2 = 12.96$, which means that number of samples can be reduced from 20 to 13.

Conclusions: The developed methodology provides reliable information about quality of managed flies by the chilled adult release system, while avoiding the use of large numbers of flies that could be destined for release purposes.

Keywords: Medfly, *Ceratitits capitata*, sample size, recovered flies, absolute flyers.

IMPROVING THE STERILE INSECT TECHNIQUE THROUGH SELECTION OF BEST PERFORMING MALES.

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Background: The Sterile Insect Technique (SIT) requires mass-production of sterile insects and mass-rearing is a selection process. There is evidence of favorable traits that have been inadvertently selected through colonization and mass-rearing, such as fast development, high fecundity, ovipositing in egg devices, and large size. However, there is also evidence of non-intentional negative selection. For example, selection for fast mating, the loss of predator evasion ability and requirements for high light intensity and temperatures. Our goal in this study was to evaluate if selection based on survival ability, predator avoidance and mating competitiveness can improve the performance of mass-reared flies for SIT application, and if this approach could be incorporated into new colony management strategies of ‘mother stocks’.

Methods: Strains of two mass-reared species were evaluated, the Mexican fruit fly, *Anastrepha ludens*, and the Mediterranean fruit fly *Ceratitis capitata*. Selection was done on host plants under field cage conditions. Wild flies were used as controls. Males selected by their survival ability, predator avoidance and wild female mating choice were crossed with mass-reared females and their offspring was reared following standard laboratory protocols. Offspring performance in the same traits was evaluated in the next generation.

Results: After one selection cycle we did not observe significant improvements in the general performance of males of the two species. Differences in survival and predator avoidance were not significant. However, the number of matings achieved by selected males were significantly greater than those achieved by non-selected (control) males.

Conclusions: The slightly better mating performance of selected males was consistently found in both species. Possible reasons for the lack of more significant effects will be discussed and new research lines will be proposed. The feasibility of incorporating such selection processes in the routine colony management of “mother stocks” is analyzed.

Keywords: *Anastrepha ludens*, *Ceratitis capitata*, mass-rearing, sterile males

NINE YEARS OF MASS-REARED OF WEST INDIAN FRUIT FLIES *ANATREPHA OBLIQUA* (MACQUART) DIPTERA: TEPHRITIDAE IN THE MOSCAFRUT COMPLEX.

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Background: *Anastrepha obliqua* is the second most economically important species for mango *Mangifera indica* L. growing in México (Aluja et al. 1987). One of the methods for controlling this pest is the Sterile Insect Technique (SIT). The Moscafrut mass rearing facility has produced this species. Since 2001, the rearing process has been optimized. In 2007 it was possible to rear the mother colony under more relaxed conditions and this improved the quality of the flies and male sexual competitiveness. In 2009 the highest competitiveness index was achieved.

Methods: The rearing conditions have involved loading cages with 60,000 pupae for the relax colony and 88,000 for release. Environmental conditions have been: $26 \pm 1^\circ\text{C}$ and 70 ± 5 RH. Larval density in the diet has been 3.7 and 5.58 larvae/ per g of diet, for relax and release rearing, respectively. The diet formulation has been: 16.33% corncob powder, yeast 6.33%, 8.66% corn flour, sugar 9.0%, 0.1% guar gum, Nipagin 0.18%, 0.33% sodium benzoate and citric acid 0.43%. The larvae are maintained for 3 days at $26-27^\circ\text{C}$, and then transferred to a room at 25 to 26°C for 3 days and finally kept at $24-25^\circ\text{C}$ until completing eight days in total. The mature larvae are separated by dissolving the diet in water. Pupation takes place at $20 \pm 1^\circ\text{C}$ for 24 hours, then the pupae are maintained at 26°C and 80% RH for 13 days. Finally, the pupae for the colony are selected and the rest is irradiated at 80 Gray and sent to release areas in Mexico.

Results: From 2001 to 2009 egg production increased from 0.416 to 1.14 eggs/cage/day. Fertility increased from 82.43% to 89.32%. Egg to larva survival has increased from 57.73% to 73.07%. Mean larva weight in the relax rearing has been 19.12 mg, and 18.71 for the release rearing. Pupae production increased from 262.649 to 2,286.885 million per year. Mean pupa weight has been 14.11 and 13.75 mg for relax and release rearing, respectively. The percentage of adult emergence and fliers gradually increased, reaching values of 86.66% and 79.82% respectively. With the implementation of the relax colony in 2007, the sexual behavior of the produced males has been successful. The RSI increased to 0.42, which has been the highest value. Competitiveness with wild flies has been acceptable with 0.63 competitiveness coefficient, above the acceptable values for the coefficient competitiveness parameter for sterile males (0.2-0.4 FAO/IAEA 2003).

Conclusions: The *Anastrepha obliqua* mass-reared system has improved during the past 9 years, accomplishing the goals of the National Campaign, both in quantity and quality.

Keywords: mass-reared, Relative Sterile Index (RSI) and competitiveness.

EVALUATION OF GENETIC SEXING STRAIN “TAPACHULA-7” (T (Y-BP⁺)-7) OF *ANASTREPHA LUDENS* (LOEW) FOR STERILE INSECT TECHNIQUE.

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Background: Genetic sexing strains (GSS) can significantly enhance Sterile Insect Technique applicability and efficiency, for Fruit Flies are becoming more relevant every day due to their operational advantages and substantial saving that represents for the action programs. A GSS based on the Y-autosome translocation system has been developed for *A. ludens*; typically pupae are brown/males: black/females. This strain has been named “Tapachula-7” and after making small-scale assessments has been identified a “good potential” for semi-massive scaling. We report here the evaluation on viability and stability in a production that is being increased.

Methods: A stock colony of the GSS “Tapachula-7” was maintained at densities of 1500 pairs of flies per cage each generation; viability parameters were measured and screened the total production recording the number of aberrants. Furthermore other cages has been maintained at densities of 3000-4000 pairs per cage, the total flies produced were revised each generation recording since F8 the crippled/half emerged flies. Production parameters were estimated in order to calculate the rearing efficiency.

The genetic study of aberrants were done by means of individual crosses, each aberrant fly was crossed with a “wild type” insect first, and then with a “black pupae” mutant. Their offspring was recorded for two generations.

Results: The stock colony “Tapachula-7” has been maintained during 19 generations and no aberrants has been found. The fertility has been maintained in 70%, which is a good value for a strain carrying a Y-autosome translocation. The viability was measured as percentage of transformations at different developmental stages: larvae-brown pupae=33.56; larvae-black pupae= 35.56, brown pupae-adult=92.34, black pupae-adult=76.59. The production was scaled and actually we maintain 5 cages with 3000 pairs each one. From these cages we obtained in average 10ml egg/day/cage and a production of pupae of 9,000/day/cage. The percentage of crippled and half merged flies was less than 2%. Only “black pupae” male aberrants have been found, when we crossed individually these insects with wild type females, the F₁ progeny was only wild type insects, but in F₂ appeared “black pupae” and wild type flies. In these aberrants insects the crosses with “black pupae” females did not produce any offspring.

Conclusions: Production and stability parameters of Genetic Sexing Strain “Tapachula-7” allow for reliable predictions on that the strain will perform properly under mass rearing conditions.

Keywords: mexicanfruitfly, genetic sexing strain, *Anastrepha ludens*, translocation, new strain evaluation, SIT

JUVENILE HORMONE TREATMENT AND PROTEIN SUPPLY IN ADULT DIET: THE CASE OF *ANASTREPHA FRATERCULUS*.

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Background: *Anastrepha fraterculus* is a major fruit pest in South America. Our research focuses on obtaining baseline data for the implementation of the Sterile Insect Technique. In this species, males have a long pre-copulatory period. We studied the effect of treating *A. fraterculus* with a juvenile hormone analogue, methoprene, which accelerates maturation in other *Anastrepha*. Because sexual development is tightly associated to protein intake, methoprene effects were analyzed under several nutritional regimes.

Methods & Results: In laboratory, we found that methoprene treatment allowed a faster sexual maturation of *A. fraterculus* fertile as well as sterile (gamma-irradiated) males. Further studies showed no differences in transferred sperm between methoprene-treated males and mature, untreated males. Protein-fed, methoprene-treated males reached sexual maturity earlier than treated males that fed on sugar, or untreated males fed on sugar plus protein. However, this was not the case for every protein source; hydrolyzed yeast showed the highest percentage of matured males. We found that a 12:1 (sugar: hydrolyzed yeast) diet is enough for methoprene to shorten the pre-copulatory period. Under an SIT program, methoprene must be delivered to millions of males at once. Dipping pupae in a methoprene solution allowed males to mature as fast as topically treated males. Including methoprene in adult diet also induced an accelerated development, but it relies on methoprene solubility in water. In field cages, we found that 6 days-old laboratory males treated with methoprene (by dipping pupae in methoprene) and fed sugar and hydrolyzed yeast at a 12:1 ratio were able to courtship, to attract of females, to mate, to transfer sperm and to induce a refractory period for mating in females as well as sexually mature wild males fed on a 3:1 diet. Young laboratory females showed lower performance (only 5% mated) than wild females irrespectively on the fact that they were treated with methoprene and fed a diet containing protein.

Conclusions: Sexual maturation of *A. fraterculus* sterile males can be accelerated by treating the flies with methoprene, but protein is needed for methoprene to act. Methoprene and protein allow laboratory males to compete for wild females in field cages. Massive delivery of methoprene is possible through feeding and dipping, and future studies should focus on massive delivery of protein and sugar. In the absence of a genetic sexing system, the fact that females do not respond as males to methoprene acts as a physiological sexing effect.

Keywords: South American fruit fly, SIT, sexing system, nutrition.

EFFECTS OF A JUVENILE HORMONE ANALOGUE, METHOPRENE, AND DIETARY PROTEIN ON *BACTROCERA CUCURBITAE* (COQUILLET): IMPLICATIONS FOR SIT.

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Background: Juvenile hormone analogue, methoprene treatment can reduce the time required for sexual maturation and access to diet including protein enhanced mating success in male melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae) genetic sexing strain under laboratory conditions, supporting their use as a treatment for sterile males within the context of sterile insect technique (SIT).

Methods: The effect of access to dietary protein (P) (hydrolyzed yeast) and/or treatment with a juvenile hormone analogue, methoprene (M), (in addition to sugar and water) on *B. cucurbitae* was assessed in the laboratory and in field cages. The males were exposed to either 1) protein and methoprene (M+P+), 2) only protein (M-P+), or 3) only methoprene (M+P-), and compared with untreated males (M-P-). The effect of methoprene and protein on mating behaviour of the four groups of males, when competing for virgin sexually mature females (M-P+), on starvation survival and on total body protein and lipids was studied.

Results: Access to a diet including protein significantly increased male performance at leks and mating success as compared to only sugar fed males. Application of methoprene had a significant effect on accelerating sexual maturity only in males with access to a diet including protein, but not in males feeding only on sugar. Combined exposure to methoprene and protein also showed a significant effect on male performance at leks. More M+P+ males called and initiated and participated in lek activities than all other types of male, attaining a higher mating success. No adverse effect of methoprene and/ or protein on starvation survival was observed when compared with the untreated males. Treating males with methoprene and/ or protein didn't inhibit female re-mating, however female mated with protein- or sugar-fed males during their first mating always preferred to re-mate with protein-fed males. Protein incorporation into the diet significantly increased the male body weight, total body carbon (TBC) and total body nitrogen (TBN) as compared to only sugar-fed males. Methoprene had no significant effect on males' TBC and TBN.

Conclusions: These studies provide strong evidence for the benefits of methoprene application and protein incorporation into the adult diet of sterile males. Treated males achieve higher sexual success, reach sexual development several days earlier, and are therefore much closer to sexual maturity when released as part of SIT action programmes after being held in the fly emergence and release facility.

Keywords: Melon fly, methoprene, dietary protein, sexual success, sterile insect technique (SIT)

IMPROVE MATING SUCCESS OF YOUNG STERILE MALES OF *BACTROCERA DORSALIS* (HENDEL) AND *BACTROCERA CORRECTA* (BEZZI).

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Background: Two tephritid fruit flies (Diptera: Tephritidae), the Oriental fruit fly, *Bactrocera dorsalis* (Hendel), and the guava fruit fly, *Bactrocera correcta* (Bezzi), are two key insect pests of fruits production causing yield loss, quality degradation and restrict international trade in Thailand and some Asia-Pacific countries. The Sterile Insect Technique, SIT, has been implement to control fruit fly species in pilot areas of mango, *Mangifera indica* Linn. (Anacardiaceae). Up-scaling from a pilot areas project to a country-wide level needed to apply SIT in a cost-effective way.

Methods: Two prerelease diet sugar, S, only and combination of sugar and protein hydrolysate, SP 3:1, were provided to sterile males during emerging, sexual separation and continuously until 2 and 3 day-old then followed with synthetic methyl eugenol, ME (1,2-dimethoxy-4-(2-propenyl) benzene), exposed and not exposed at releasing age 2 and 3 days. Mating competitiveness experiments were conducted as 2x2 factorial in RCBD, (S without ME); (S with ME); (SP 3:1 without ME) and (SP 3:1 with ME) over five replications. Sterile males (2+n and 3+n, where n = 1-15 testing day) of *B. dorsalis* were set up; 50 sterile males and 50 wild males, 23 day-old, were completed for 50 wild females, 21 day-old. But the observation day for *B. correcta* was 18 days. The same number of *B. correcta* sterile and wild males were designed for wild females competition as *B. dorsalis* but the wild females was 37 day-old.

Results: *Bactrocera dorsalis* and *B. correcta* showed additive interaction of ME and both S and SP 3:1 as prerelease diet via significantly higher percent of mating. RSI of sterile males given S with ME and SP with ME presented higher the percentage of mating significantly than prerelease diet feeding without ME exposure.

Conclusions: Based on our result, we would recommend for weekly sterile male released program. Sterile males should provide sugar/protein (3:1) into adult eclosion box for 2-3 days then they would be exposed to ME in the morning of release day.

Keywords: *Bactrocera dorsalis*, *Bactrocera correcta*, mating success, prerelease diet, methy eugenol.

EFFECT OF TRANSPORTATION AND SHIPPING IN THE QUALITY OF THREE MASS REARED SPECIES IN MEXICO.

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Background: The transport and shipment of fruit flies to be used in the Sterile Insect Technique (SIT) have to consider two basic aspects: 1) administrative and operational requirements and 2) Handling of biological material which includes type and conditions of packaging, transportation, distance traveled (shipping time), management in emergency areas, system for emergency and finally the release of biological material. The conditions that remain may affect the quality of adults produced, but even with optimal management, shipping time affect the emergence and flight ability of adults and negative effects in the performance of insects, competitiveness of flies or search capacity of parasitoids.

Methods: Moscafrut Facility located in the south-east of Mexico have a mass rearing of *Anastrepha ludens*, *A. obliqua* and *D. longicaudata*. The biological material produced (sterile pupa or parasitized pupa) is separated from the substrate of pupation and packed in sausage bags, which are placed in cardboard boxes are then transported by air to the International Airport of Mexico City where they are transshipped to packaging and releases centers in the Northern states. During the packing, the pupae are maintained under hypoxic conditions for the sterilization of the flies, preventing the emergence of the material during the transportation. To measure the effect of shipping on the quality of both flies and parasitoid we compare the information generated during three years at origin and destination.

Results: The transportation and shipment of the pupa to the destination resulted in a significant loss in quality parameters for each species distributed in the different states of Mexico. To *Anastrepha ludens*, the emergency of 92.78% and flight ability of 90.37% were reduced by 6.78% and 11.21% respectively. For *A. obliqua*, sent to two states, the values were 91.90% and 86.51% with 2.63 and 6.34% loss for emergency and flight ability, respectively. The emergence of the parasitoid, *D. longicaudata*, was decreased by 9.14% at arrival with an initial value of 60.55%. The quality was affected significantly by the time of hypoxia, the effect being most noticeable when observing differences in travel times within a single destination.

Conclusions: To minimize the effects on the quality of mass-produced insects, logistics must be organized in order to reduce the time of hypoxia.

Keywords: emergence, flight ability, hypoxia, *Anastrepha*, *Diachasmimorpha*

OPTIMUM DOSE OF GINGER ROOT OIL TO TREAT STERILE *CERATITIS CAPITATA* (WIEDEMANN) (DIPTERA: TEPHRITIDAE) MALES.

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Background: The sterile insect technique (SIT) is widely used as part of an integrated approach to reduce field populations of the Mediterranean fruit fly (*Ceratitidis capitata*, Diptera: Tephritidae). Aromatherapy based on exposure to ginger root oil (GRO) volatiles is known as a method to significantly improve the sexual performance of sterile medfly males, and is being used in many mass-rearing facilities around the world. However, the optimum dose of GRO is not well defined.

Methods: This work evaluated in laboratory cages four different doses of GRO and a control (0, 0.1, 0.25, 0.5, and 0.75 ml/m³), and two different methods to hold flies prior to release (paper bags and plastic cages). The objective was to find the lowest dose that provides optimal improvement in mating performance of sterile males when competing with wild males for wild females and optimal reduction in fertility of wild females. Egg hatch, copula duration, the Relative Sterility Index (RSI), and a Competitiveness (C) value (based on RSI) were calculated for each treatment to assess male sexual performance and induction of sterility.

Results: The method used to hold flies did not influence the aromatherapy effect. The mean time spent by wild females in copula with wild males was significantly longer than with sterile males for all treatments, except when sterile males were treated with 0.1 ml of GRO/m³. Among all doses studied, the dose 0.1 ml of GRO/m³ reached the highest levels for both RSI and induced sterility, and was not statistically different from the 0.25 and 0.5 GRO/m³ doses.

Conclusion: The flies can be treated aromatically with ginger root oil in either paper bags or ventilated plastic cages. It is recommended to apply the lowest dose, 0.1 ml of GRO/m³, because it showed the best cost/benefit ratio when used in the Mediterranean fruit fly SIT programme in the San Francisco River Valley, Brazil.

Keywords: Aromatherapy, Sterile insect technique, Mediterranean fruit fly, Medfly, GRO.

FIELD CAGE STUDIES ON MATING ISOLATION BETWEEN SPECIES WITHIN THE *BACTROCERA DORSALIS* COMPLEX.

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Background: There is increasing demand, world wide, to address the issue of fruit fly species complexes, i.e. fruit fly species with distinct similarities that have been grouped together. There is concern that some of these insect lines are not separate species but geographical variants. Their uncertain taxonomic status is having significant implications for international trade and the efficacious use of the Sterile Insect Technique (SIT).

Methods: Interspecific crosses between the following species were assessed: *Bactrocera dorsalis*, *B. philippinensis*, *B. invadens* and *B. carambolae*. 1) No-choice laboratory mating tests. Fly species were reared under identical conditions, sexed within 24h of adult emergence and housed in small cages with food and water. Five male flies of one species and 5 females of another species were housed in each cage and the experiment was replicated three times. Data on the numbers of eggs oviposited, egg fertility, egg-pupa recovery and the sex-ratio of F1 hybrids were recorded. 2) Field cage mating tests. Flies were segregated by sex and “painted” with a dot of water-based paint when sexually mature. Each species / gender was painted with a different colour. Twenty males of each of the two species being tested were released into each of four field cages and, 30min later, 20 females of each of the two species were released. When mating couples formed the time of coupling was recorded, the mating couple was removed and identified through their colour code.

Results: Under no-choice laboratory mating tests all attempted hybridisations produced viable offspring. However, under simulated field conditions various degrees of mating isolation between species were observed.

Conclusions: Implications of these observations are discussed in regard to species separation, international trade and SIT.

Keywords: *Bactrocera dorsalis*, species complex, mating behaviour, interspecific hybridisation

Session 6
Area-Wide & Action Programs

HAWAII AREA-WIDE FRUIT FLY INTEGRATED PEST MANAGEMENT PROGRAM: A MODEL SYSTEM.

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Background: In Hawaii, fruit flies limit development of a diversified fruit and vegetable industry, require export fruits to undergo expensive quarantine treatments, and provide a reservoir for pest introduction into the mainland United States. Hawaii has four species that have become established, three of which (Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), melon fly, *Bactrocera cucurbitae* (Coquillett), and oriental fruit fly, *Bactrocera dorsalis* (Hendel)) are considered major pests of agriculture worldwide. When these pests are introduced into the U.S. mainland, they often require large-scale eradication programs, often at great public expense. In California, where the total value of the fruit and vegetable industry has been estimated to be more than \$14 billion annually, the California Department of Food and Agriculture has estimated that an established infestation of Mediterranean fruit fly would cost from \$855 million to \$1.4 billion during the first year of establishment.

Methods: A partnership Hawaii Area-Wide Pest Management (AWPM) project implemented by ARS resulted in the first successful program to control fruit flies that have been devastating Hawaiian agriculture for almost 100 years. The control system based on a combination of techniques (sanitation, protein baits, male annihilation, and SIT), developed primarily by ARS, have been adapted and coordinated into an IPM initiative specifically designed to work in Hawaii's environment. A hallmark of the program has been a network of partnerships involving ARS, the Hawaii Department of Agriculture, the University of Hawaii Cooperative Extension Service and local communities, with the support of APHIS and other research, regulatory and government agencies.

Results: New technologies transferred to farmers included new monitoring systems, new protein bait systems, and new male annihilations treatments. The 2,079 signed cooperating growers in the program, representing 6,798 acres, 491 farms, across five islands so far-Oahu, Hawaii, Molokai, and Maui--have already been able to cut conventional pesticide use by 75-90%, and reduced fruit fly infestation from 30-40% to less than 5%. Small farms are now growing crops they had previously been abandoned due to fruit fly damage.

Conclusions: An economic assessment determined that the Hawaii AWPM program was easy to use, and initial economic benefits were estimated at \$2.6 million per year and projected. Even using the most conservative economic analysis, without including the possible benefits category, the rate of return still came to 27%, according to a cost-benefit study. The success of the Hawaii AWPM program has had international impacts on fruit fly management, as many other countries facing similar problems. Researchers and officials from People's Republic of China, the Commonwealth of the Northern Mariana Islands, French Polynesia, Guam, Senegal, and Taiwan, among others, have expressed interest in or adopted the program as a model for fruit fly suppression.

Keywords: Mediterranean fruit fly, oriental fruit fly, melon fly, area-wide integrated pest management.

STATUS OF *BACTROCERA INVADENS* MANAGEMENT IN AFRICA: RECENT ADVANCES, SUCCESSES AND CHALLENGES.

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Background: *Bactrocera invadens* was first detected at the Kenyan coast in 2003 and later described as a fruit fly pest that was completely new to science. It is probably native to Sri Lanka and has rapidly expanded its geographical range, now reported from over 28 African countries. The pest attacks numerous cultivated and wild host plants but mango is the primary host with damage ranging from 40-80%. Quarantine restrictions due to the insect have significantly restricted access to lucrative export markets abroad. For example, exports of host crops of *B. invadens* are already banned to Seychelles, Mauritius and South Africa; and trade of several horticultural produce between Africa and the US has been severely hampered.

Methods: Since its detection, several international and national partners have been involved in investigations related to its distribution, abundance, host range and interaction with native *Ceratitis* species. Morphometrics and molecular studies on the insect have been carried out to unravel its relationship with other *Bactrocera* species as well as its invasion history. Several attractants and fungal-based biological control agents have been evaluated for suppression. Assessments of the role of the weaver ant *Oecophylla longinoda* in significantly decreasing damage, as well as farmer perception of the ant, have been carried out. Exploration for natural enemies in the putative aboriginal home has been undertaken and parasitoids have been introduced into Africa from established laboratories from the USA. Releases of *Fopius arisanus* has commenced in several countries. Post harvest disinfestations trials on citrus and avocado has been initiated jointly with the private sector to recoup export markets.

Results: A comprehensive assessment of the distribution of *B. invadens* in Africa and potential range of expansion based on ecological niche model will be presented. Field observation depicts rapid displacement of native *Ceratitis* species. Host range studies showed that the pest attacks over 40 plants species. Morphometric analysis shows clear relationships with *Bactrocera kandiensis* and ongoing DNA barcode analysis will be presented. Analysis of invasion history using microsatellite markers revealed 2 points of invasion in Africa – Kenya and Benin. Potent attractants (e.g. GF-120 and Mazoferm, local waste brewer's yeast) and fungal-based biopesticide have shown considerable potential for field suppression. Field releases of *Fopius arisanus* have commenced in several countries with significant recoveries indicating potentials for establishment. Results from ongoing cold disinfestations trials on avocado and citrus will be discussed.

Conclusions: An IPM package based on the use of protein bait, male annihilation technique, biopesticides, biological control (ants and parasitoids) and orchard sanitation is being advocated, and progressively implemented by growers for access to both domestic and international markets. However adoption seems far fetch from the reality and challenges to implementation across Africa will be discussed.

Keywords: *Bactrocera invadens*, invasion, spread, bioecology, suppression.

PATAGONIA ARGENTINA. FRUIT FLY FREE AREA.

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Background: The Patagonian Protected Region is a vast area of 835.000 km² and about 1.7 million inhabitants. There are 150.000 hectares of irrigated valleys which form a real oasis within the typical semiarid plain landscape. These valleys are suitable for the production of vegetables, pome, stone and fine fruits (berries), all of which are in very good condition for export. The Region produces 1,000,000 tons of apples, 600,000 of pears (which represent the 85% of the country's production) and 100,000 tons of stone and fine fruits.

Methods: The whole Region is naturally isolated by the Andean Range to the west, the Atlantic Ocean to the east and the Colorado and Barrancas Rivers, to the north. In addition to this, 13 terrestrial inspection posts, 13 airports, 1 railroad control post and all the seaports of the Patagonian coast protect the area in order to prevent from any eventual re-infestation from the northern part of the country.

Because of the presence of medfly in some urban areas, both Private and Public sectors in Patagonia decided, in 1997, to face the eradication of the pest in order to prevent quarantine restrictions imposed by some countries. After many years of hard work, combining regulation of incoming articles, Sterile Insect Technique, and cultural and chemical control, Patagonia was finally recognized as free of Fruit Flies by USDA/APHIS, by December 2005.

The Program runs a 2.500 traps network, serviced every week, which demands 30 people working as trappers and samplers and identification laboratories. Control activities are concentrated, at present, on the implementation of the SIT through a Preventive Release Program over some urban areas where chances of a re-infestation are higher.

Results: In case an outbreak occurs, an Emergency Plan, including focused control and regulatory activities, is immediately put into action. Since the eradication of the pest, two outbreaks occurred, one in 2006 and the other one in 2009. Both have been satisfied controlled and finally eradicated.

Conclusions: This situation enabled Patagonia export to the United States for the fifth consecutive season without quarantine treatments.

ECONOMIC EVALUATION OF THE NATIONAL CAMPAIGN AGAINST NATIVE FRUIT FLIES IN SIX MEXICAN STATES (1994-2008).

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Background: The National Campaign against native fruit flies (*Anastrepha spp.*) was established by the Mexican Government in 1992 to prevent the negative effects of this pest in fruit loss in the field and quality of products, and through it, facilitate farmers the commercialization of their fresh fruits both in national and international markets. Thanks to the Campaign, several Mexican states and regions have been recognized as free areas (Baja California, Sonora, North & Central Sinaloa, Chihuahua and North of Zacatecas) and low prevalence areas of native fruit flies (South of Sinaloa, Nuevo León, Tamaulipas & Aguascalientes). This has facilitated observed increments in production and exports of Mexican fruits in the states where the Campaign has been operating. The different species of native fruit flies can cause up to 20% fruit loss in the field of sweet citrus and mango, 15% of guava and 30% of “mamey” and “chico zapote” if there were no phytosanitary measures implemented against this pest.

Methods: Economic impacts were estimated through a retrospective model that was designed using as a guideline the “Cost-Benefit Analysis Model: A Tool for Area-Wide Fruit Fly Management” developed by FAO/IAEA. The designed model considered historical data of the observed variables, program costs and benefits (direct & indirect) for a 15 years period, to estimate the economic indicators: benefit-cost ratio (B/C), net present value (NPV), internal rate of return (IRR) and pay-back period.

Results: Estimated economic indicators for each of the six evaluated states showed a B/C ratio much higher than one, positive NPV, very significant IRR and a pay-back period in only one year. A total investment in the Campaign of US \$188 million for all six states during a 15 year period (6 for the state of Guerrero), generated direct and indirect benefits of US \$1.8 and 1.5 billion, respectively. Benefits for each state were: US \$13 and 12 million, respectively, for Baja California, US \$303 and 143 million for Guerrero, US \$208 and 424 million for Nuevo León, US \$578 and 340 million for Sinaloa, US \$380 and 158 million for Sonora and US \$347 and 456 for Tamaulipas. For the mango produced in four of these states, direct benefits rose to US \$960 million and indirect ones to US \$548 million, while for the sweet citrus species cultivated in the six states, US \$1.3 and 1.0 billion, respectively.

Conclusions: Based on the obtained economic indicators, the decision of the Mexican government of establishing the National Campaign against Native Fruit Flies was rational from the economic point of view, since it has been significantly profitable to the six evaluated Mexican states during the analyzed period. Through this Campaign, Mexico has benefited from a growing production and exports of fruit, maintained employment in the agricultural sector and saved negative impacts to the environment. Keeping Mexico free of the Mediterranean fruit fly (a non native species) has been a strategic objective of the Mexican Government and a key in allowing the economic success of the National Campaign against Native Fruit Flies.

Keywords: Native fruit flies, economic indicators, benefit to cost ratio

NEW TECHNOLOGY ON STERILE INSECT TECHNIQUE FOR FRUIT FLIES ECLOSION AND RELEASE IN MEXICO.

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Background: The National Service for Plant, Animal and Forest Health of Mexico (SENASICA) have invested in building new facilities for packing, eclosion and aerial release of chilled adults of fruit flies to improve the Sterile Insect Technique (SIT).

Methods: The technicians of Fruit Flies Program with the support of private enterprises have developed and improved new containers for packing and eclosion pupae and adults, towers type called "México" and "V&Z", build with screened levels for lateral ventilation. With plastic accessories for pupae eclosion and to keep the puparia and residues for a clean free of paper process. A kind of plastic accessory increases surface and capacity to emerge a larger amount of pupae-flies as an easier alternative to "Worley" towers and PARC boxes containers.

For feeding and drinking water, fruit flies in those new containers use a fruit flies balanced diet, 90% sugar and carbohydrates and 10% vegetable proteins, coming from a mixed of amaranth, peanut, egg powder, corn and sugar. Water is provided in a sponge or a cotton padded fabric. This is an alternative to the food made of Agar, sugar and water.

For aerial release automatic machines are used with intelligent refrigerated boxes to keep temperature between 0-5°C and humidity below 50%, for an optimal chilled adult flies release process. Those machines are provided with a conveyor (50cm. wide) working in transversal way to the axis of the plain. The released fly density per hectare is regulated by the speed of the conveyor and size of the gate of airplane chute. The precision release is made by aerial navigation system AGNAV, GPS. All process are recorded and monitored by an interactive Web site.

Results and Conclusions: The new technologies for SIT have been applied since 2000 and have been released around 104,000 million of sterile flies of the *Anastrepha* genus and 61,200 million of *Ceratitis capitata* (Wiedemann). All this have contributed to achieve the goals of the Fruit Fly Programs of Mexico, with the creation and expansion of free and low prevalence areas around 49% of Mexican territory and in the containment and eradication of Medfly in Mexico and Guatemala border.

Keywords: Medfly, fruitfly, packing and eclosion, balanced diet , aerial release,

NATIONAL FRUIT FLY CONTROL AND ERADICATION PROGRAM (PROCEM) ARGENTINA.

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Background: The National Fruit Fly Control and Eradication Program was created in Argentina in 1994, with the aim to suppress and eradicate the species *Ceratitis capitata* (Medfly) and *Anastrepha fraterculus* (South American Fruit Fly), according to the agrobiological conditions of each region. Considering that the production of fruits and vegetables accounts for 20% of total Agricultural Gross Domestic Product (pome and stone fruits: 126,952 ha; citrus fruit – excluding lemon–: 96,965 ha; grapes: 226,450 ha), the main objectives of the Program are to reduce economic losses caused by fruit flies and to facilitate the access of fruit and vegetable products to international markets.

Methods: The Program is currently implemented in 910,000 hectares, with the support of 1,060 professionals and technicians, developing the following activities: Surveillance (setting of traps and fruit sampling to monitor *Anastrepha fraterculus* and *Ceratitis capitata*, and to detect exotic fruit flies, like: *Ceratitis* spp., *Anastrepha* spp., *Toxotrypana curvicauda*, *Bactrocera* spp., *Rhagoletis* spp. y *Dacus* spp.), Control (sterile insect technique, chemical control methods, biological control activities, cultural practices and legal control), SIT production (there are two rearing facilities, located in the Provinces of Mendoza and San Juan, which provide the sterile material to the areas under official program), Quarantine Protection System (phytosanitary barriers inspection and control vehicles and loads, 24 hours a day, all the year, using organic material detectors and/or detector dogs), Quarantine Treatment Facilities (approved quarantine treatments are applied to fruit fly host products, prior to their entry into the protected areas), Quality Controls, Training, Diffusion and Communication.

Results & Conclusions: The program has achieved different status of plague, including: Fruit Fly-Free Areas (Central and Southern Oases of Mendoza Province and the Patagonia Region), Areas with Low Prevalence of *C. capitata* and Free of *A. fraterculus* (Northern and Eastern Oases of Mendoza Province), Areas with Low Prevalence of *C. capitata* and Under Control of *A. fraterculus* (Calingasta's Valley of San Juan Province), Areas Under Control of *C. capitata* y *A. fraterculus* (Cultivated Valleys of San Juan Province) and Uncontrolled Population subject to Monitoring Surveys (Monte Caseros-Colón, Provinces of Corrientes and Entre Ríos).

STUDIES ON SMALL MODEL AREA-WIDE CONTROL OF THE ORIENTAL FRUIT FLY FOR WAX APPLE IN TAIWAN.

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Background: The area-wide (AW) control of the oriental fruit fly (*Bactrocera dorsalis* (Hendel), OFF) was tested, using the conditions defined by Lindquist (2000), in Juan-Nan area of Mei-Shan township, Chia-Yi county, where wax apple is the major crop with significant economical importance. Growers in this area show a common interest in wax apple production, hence are highly cooperative in managing the fruit fly population.

Methods: All available OFF control techniques have been utilized, e.g., methyl eugenol bait for male annihilation; protein bait for female fly control; bagging of the fruits; and thorough field sanitation in all wax apple orchards. A well organized OFF density monitoring program was established with 12 monitoring sites, which covered 500 ha testing area. By using 3D aerophotos display system, control planning and farmer education were conducted under the AW control scheme.

Results: Comparing OFF density from individual monitoring sites in 2005 and 2006, the high density sites almost the same to conjecture that sites are hot spot possibility. The similar result reappears next year. In two years of AW control practice, the OFF population dropped below the economic threshold and fruit production was in excellent quality with a financial gain of NT\$ 4 million (\$ 122,000) in 2005. In 2006, the OFF population density was further reduced 50% from that of 2005, and the estimated economic gain on fruit value combining with the saving of pest control cost exceeded NT\$ 9 million (\$ 286,000). The evaluation of the AW control program also included a survey of farmers' inputs, which concluded a score of 4.0 in the scale of 1 to 5. The control practice covering 500 ha was considered small in the AW control model for OFF, as if did not have any buffer zone established. Despite the high mobility of OFF, no serious re-invasion problem from the adjacent area occurred due to the scarcity of alternative hosts in that area.

Conclusions: This study, though confirmed the AW control requirements as proposed by Lindquist, recommends the requirements supplemented with such additional elements as effective control tactics, continuous farmer education, new and available technologies.

Keywords: *Bactrocera dorsalis*, Area-wide control, Wax apple, Small acreage model.

DEVELOPING, IMPLEMENTING AND ECONOMIC ANALYSIS OF AREA-WIDE INTEGRATED MANAGEMENT OF MANGO FRUIT FLY, *BACTROCERA DORSALIS* (HENDEL) IN SOUTH INDIA

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Background: The fruit fly, *Bactrocera dorsalis* (Hendel) is a major pest of mango (*Mangifera indica*) causing on an average 27% loss in India. Additionally it has been an impediment in exports. So, an Integrated Management of Fruit Flies (IMFFI) project was initiated in India in 2001, as a joint collaboration between Indian Council of Agricultural Research (ICAR, New Delhi) and Department for International Development (DFID, UK). The main objective was to develop an integrated management (IM) of the mango fruit fly *B. dorsalis*.

Methods: The IM was developed and standardized, between 2001 and 2005. Between 2006 and 2009, the IM was transferred to mango growers on an area-wide basis. The strategies investigated were male annihilation using methyl eugenol, field sanitation, bait application, visual (colour lures) and use of *Oscimum* extract. The economic impact of the IM was assessed using a semi-structured questionnaire in a major mango belt in South India.

Results: It was found that methyl eugenol impregnated plywood (with dichlorvos as toxicant) placed in a plastic container (500ml capacity) with two circular vents (1.5cm diameter) served as an ideal cost-effective trap [Cost per trap= 0.5 Euro]. It was worked out that 6-8 traps/acre could serve both as a surveillance tool and male annihilator (MA). A series of experiments with several indigenous baits showed that jaggery or banana pulp at 10% (mixed with a toxicant like malathion) was on par with protein hydrolysate, as splashes and could be used in IM as bait application technology (BAT). The number of BAT splashes were standardized to 40/acre, at the rate of 50-100ml bait solution per tree trunk (given two feet above the ground). Likewise, cultural approach of regular sanitation (weekly removal and destruction of fallen fruits) showed additive control. These measures, viz, MA, BAT and sanitation on an area-wide basis gave excellent control (95-100%). These areas were in pockets within a contiguous mango belt in south India between 12^o E, 79^o N. The impact analysis showed that these farmers realized at least 20-40% yield increase, with a cost: benefit ratio ranging from 1:4 to 1:20, depending on the commercial value of the mango variety. Visual and *Oscimum* lures were not effective.

Conclusions: Farmers expressed high satisfaction with the area wide IM of fruit flies. So it is envisaged, to cover IM of fruit fly on a wider area, in 2010 and 2011 with financial support from ICAR and NABARD (National Bank for Agricultural and Rural Development, India.). This will further strengthen the adoption of fruit fly IM in mango in India and South Asia where *Bactrocera* spp are a problem.

Keywords: *Bactrocera* spp, Mango, area-wide, male annihilation, baits, integrated management, India

Session 7
Natural Enemies & Biocontrol

INSECTICIDAL COMPOUNDS SECRETED BY ENTOMOPATHOGENIC FUNGI SHOW PROMISE FOR *CERATITIS CAPITATA* (WIEDEMANN) CONTROL.

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Background: The control of *Ceratitis capitata* is usually performed with protein bait sprays incorporating chemical insecticides that have adverse effects on humans, non-target organisms, and environment. For that, in the last decade, there has been an increasing interest for the so known natural insecticides for medfly control, including those from higher plants and microorganisms. Among microorganism, naturally occurring entomopathogenic fungi (EF) not only are excellent candidates for medfly microbial control but also a new poorly explored source of novel insecticidal compounds of natural origin

Methods: The crude soluble protein extract (CSPE) in liquid media of 130 isolates from our collection of autochthonous strains of EF obtained from the soil, insect and phyllo plane belonging to several EF species, with emphasis in *Metarhizium anisopliae*, *Beauveria bassiana*, have been evaluated for insecticidal activity against newly emerged medfly adults when administered per os. The active fractions of the CSPE have been purified by liquid chromatography and gel electro-elution. The active protein fractions have been characterized and their acute, chronic and sublethal reproductive effects on medly adults evaluated.

Results: To now, only three out of the 130 CSPEs evaluated have shown insecticidal activity against medfly adults. Among them, the CSPE of *M. anisopliae* EAMa 01/58-Su strain is the most active. The mortality in flies fed with this CSPE exhibits a dose and time-related response, a result likely due to a progressive deterioration on the fly midgut after ingestion of the extract. Four monomeric proteins from this crude extract have been purified by liquid chromatography and gel electro-elution. Although all four monomers seem to be involved in the insecticidal activity of the CSPE, the 15 kDa and the 11 KDa proteins appear largely responsible for the observed insecticidal effect. Besides, the activity of the CSPE of this isolate can be highly enhanced (by seven fold) by manipulating the nutrient conditions of the liquid medium (carbon and nitrogen sources and ratios). Likewise, a reduction of female fecundity levels during the first days after the treatment has been found in CSPE challenged flies.

Conclusions: We have presented evidence that the CSPE of *M. anisopliae* 01/58-Su strain should be considered as a new tool for biocontrol of *C. capitata* adults that can be combined with an attraction bait-system to increase its efficiency while minimizing the impact on beneficial insects.

Keywords: natural insecticides, medfly, fungal proteins, metabolites, *Beauveria*, *Metarhizium*, baits, fungal extracts, macromolecules, toxicity

CERATITIS CAPITATA (WIEDEMANN) SUSCEPTIBILITY TO CYT1AA TOXIN FROM BACILLUS THURINGIENSIS (BERLINER).

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Background: The Mediterranean fruit fly, *Ceratitis capitata*, is one of the major threats to fruits crops worldwide. Current control methods rely mostly on synthetic insecticides. Their impact on human health and the environment along with the development of resistances make necessary the implementation of sustainable control methods. *Bacillus thuringiensis* (Bt) based-products should be a good alternative since they have been used for decades for controlling economically important pests. Though δ -endotoxins are highly toxic and specific against many insect pests, including dipterans, previous works performed in our group have reported that *C. capitata* is not susceptible to spore and crystal (S+C) mixtures (containing δ -endotoxins) obtained from a wide array of Bt isolates. The goal of this work was to overcome the reported inefficiency of Bt S+C mixtures against *C. capitata* by the administration of solubilised and/or activated δ -endotoxins.

Methods: Our first approach was to test the *in vitro* solubilized crystal protoxins obtained from cultures of 35 native selected strains and 5 standard strains. Proteolytic activation of δ -endotoxins produced by the most toxic strains was also tested; the crystals were *in vitro* solubilised and thereafter incubated with gut extracts from 3 insect species (*C. capitata*, *Sesamia nonagrioides* and *Culex pipiens*).

Results: Bioassays on neonate *C. capitata* larvae showed significant sublethal effects of solubilised protoxins at 20 $\mu\text{g}/\text{cm}^2$ on one standard and two native strains, all belonging to the Bt serotype *israelensis* (Bti). Protein electrophoresis revealed that protoxin activation was strongly dependent on the source of proteases. Dose-response assays showed that *in vitro* proteolytic processing of Bti protoxins increased lethal effects on *C. capitata* neonate larvae, being significantly higher when protoxins were activated with *Culex pipiens* proteases, a dipteran species highly susceptible to Bti. LC_{50} of *C. pipiens* activated Bti toxins was 31.26 $\mu\text{g}/\text{cm}^2$. Additionally, the use of a recombinant Bt strain allowed the identification a single δ -endotoxin, Cyt1Aa, responsible for the lethality found on *C. capitata* larvae. LC_{50} of this protoxin after solubilisation was: 4.93 $\mu\text{g}/\text{cm}^2$.

Conclusions: We have shown that *in vitro* emulation of events from Bt mechanism of action may increase the activity against recalcitrant pests such as *C. capitata*. The identification of a single δ -endotoxin with larvicidal activity may be the basis for new engineered Bt strains effective on *C. capitata* control.

Keywords: *Ceratitis capitata*, *Sesamia nonagrioides*, *Culex pipiens*, proteases, bioassays

COMPATIBILITY OF A NEW ATTRACTANT-CONTAMINANT DEVICE CONTAINING *METARHIZIUM ANISOPLIAE* WITH MEDFLY SIT PROGRAMS.

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Background: The Sterile Insect Technique (SIT) is one of the most useful strategies to control the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), medfly. However, new or improved eco-friendly control methods are required to synergise or complement SIT programs. One of the new tools currently being studied is the use of entomopathogenic fungi. Recently, a new *C. capitata* attractant-contaminant device, containing *Metarhizium anisopliae* (Metschnikoff) Sorokin, developed by our group, showed a high field efficacy on this citrus pest. The aim of this work was to evaluate the compatibility of ongoing SIT programs in the Comunidad Valenciana with this “attractant -contaminant” technique. The combination of these two techniques would be feasible providing that at least the sterile males do not show a higher susceptibility to *M. anisopliae* than the wild population, and that the mating performance of the sterile male is not affected.

Methods: Susceptibility of *C. capitata* Vienna 8 males to *M. anisopliae* was evaluated by topical application and compared with that previously obtained from a *C. capitata* laboratory strain, annually refreshed with wild medflies (WTL strain). A comparative mating performance bioassay between sterile and WTL medfly males exposed to attractant-contaminant devices (fungus contaminated or fungus-free) was also carried out. One day after the exposure, healthy virgin WTL females were allowed to mate with each male group. Mating pairs were individually collected during three hours and the number of mating and their duration recorded. Mated medfly females were kept in separated cages for two days, and then allowed to remate with non-treated WTL males. Then, remated females were maintained individually in separated cages to determine the fecundity and fertility.

Results: The pathogenicity bioassays established that the susceptibility of *C. capitata* Vienna 8 males to our *M. anisopliae* strain was similar to that showed by the WTL strain, and did not impair the percentage of mating. However, the duration of the copula increased significantly for copulating WTL male treated with *M. anisopliae*. No significant differences on fecundity were found between females mated with the *M. anisopliae* treated and not treated males, but the fertility of remated females was significantly higher for those mated twice with males of the WTL strain, despite of the treatment.

Conclusions: *M. anisopliae* does not adversely affect any of studied parameters linked to medfly mating performance, suggesting that the “attractant-contaminant” technique would be compatible with SIT programmes.

Keywords: Medfly, entomopathogenic fungi, biological control

PEST, PARASITOID AND FRUIT INTERACTIONS IN BIOLOGICAL CONTROL OF THE OLIVE FRUIT FLY IN CALIFORNIA.

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Background: Olive fruit fly, *Bactrocera oleae*, has become a key pest in olives, *Olea europaea*, since it was first discovered in California in 1998. Biological control is an economical alternative for costly bait sprays in commercial orchards of olives grown for canning and oil. Several years of augmented releases of an imported parasitoid, *Psytalia humilis*, has resulted in successful parasitism of olive fruit fly, but the parasitoid has not yet become established.

Methods: The life cycle of olive fruit fly and *P. humilis* was studied in the laboratory, greenhouse, and field. Survival of the adult and immature stages of olive fruit fly and the adult stage of *P. humilis* was investigated at constant temperatures (15-25°C) and humidities (35-65%) in laboratory incubator tests, and in two greenhouse climates with fluctuating diurnal (32-36°C, 31-37% hot vs. 26°C, 61-63% cool) and nocturnal conditions, and with and without honey for food and water. Seasonal field temperatures and humidities were monitored with data loggers. Olive fruit fly adult infestations were determined in the field with yellow sticky traps baited with ammonium carbonate baits and pheromone lures. Fruit samples were collected to determine natural larval infestations and parasitism after parasitoid releases.

Results: Olive fruit fly and *P. humilis* required cool temperatures, high humidities, and food and water for prolonged survival (\approx 6 months for host) in laboratory and greenhouse tests. Life span was greatly shortened by high temperatures, low humidities, and the absence of food and water. The observations were consistent with high populations of the pest found in cool coastal areas and low numbers that occur in dry, arid inland valleys. The parasitoid was effective for biological control (\leq 100% parasitism) when fruit infestations were high such as in coastal olive growing areas. Non-establishment of the parasitoid was related to the lack of infested olive fruit remaining in the trees during winter. The major factors that caused infested fruit suitable for development of *P. humilis* to drop from trees were high larval infestations and occasional freezing winter conditions. The parasitoid could survive (\approx 4 months at 15°C) and reproduce (10°C) in the host during winter. Olive fruit fly larvae were found to complete development in immature fruit 1 cm long increasing availability of host between annual crops.

Conclusions: Successful biological control of olive fruit fly in California is dependent on the presence of infested fruit which is destroyed by high larval numbers and inclement weather. The parasitoid is long-lived and capable of overwintering as adults, but the host may be unavailable due to fruit condition.

Keywords: Olive fruit fly, *Psytalia humilis*, biological control, olives

CONSERVATION BIOLOGICAL CONTROL STRATEGIES IN CITRUS AS A TOOL TO REGULATE MEDFLY POPULATIONS.

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Background: In recent years, emphasis has been placed on implementing environmentally friendly methods to control medfly. Within this context, biological control measures based on predators have hardly been used. In medfly there are three developmental stages that are susceptible to being preyed upon by ground-dwelling predators: late third instars larvae, which jump from the fruit to pupate underground, pupae and teneral adults, which remain on the soil until they are able to fly. We report here on the importance of these ground-dwelling predators in citrus groves and their influence upon *C. capitata*.

Methods: A 3-years study of the ground-dwelling predator assemblages associated to citrus orchards has been conducted in Valencia Region (Spain) using pit-fall traps. Activity-density patterns, community structure, species richness and abundance parameters of these assemblages were evaluated. Functional responses against the medfly developmental stages susceptible of being preyed upon were obtained under laboratory conditions for the prevalent predator species of each group. A *C. capitata* specific PCR marker was designed and used to assess medfly predation by means of predator gut-content analysis.

Results: This work reveals the presence of a rich and abundant complex of ground-dwelling polyphagous predators inhabiting the ground surface of citrus orchards throughout the year. Indeed, 17,526 ground-dwelling predator specimens belonging to 110 different species were captured. The prevalent predators found were the lycosid *Pardosa cribata* (Araenae), the ground beetle *Pseudoophonus rufipes* (Coleoptera: Carabidae) and the earwig *Forficula auricularia*. All these prevalent predators were able to prey upon *C. capitata* under laboratory conditions. *Pseudoophonus rufipes* was the most efficient predator, while *F. auricularia* was the least. *Pseudoophonus rufipes* preyed mainly upon pupae, with an estimated attack rate of 3.07 d⁻¹ and a handling time of 0.048 d, *P. cribata* used teneral adults as the main prey, with an estimate attack rate of 0.771 d⁻¹ and a handling time of 0.051 d, and *F. auricularia* showed the highest preference for third-instar larvae, with an estimated attack rate of 0.269 d⁻¹ and a handling time of 0.065 d. The carabid *P. rufipes* turned out to be the most efficient predator under field conditions, with 23% specimens testing positive after PCR gut-content analysis, while the proportion of *P. cribata* testing positive was 5%.

Conclusions: Ground-dwelling predators could play an important role on regulating *C. capitata* populations. A challenge for the future will be how to enhance their populations, to increase their efficiency against this pest, and consequently, to incorporate them in current citrus conservation biological control strategies.

Keywords: Ground-dwelling polyphagous predators, medfly, functional response, PCR gut-content analysis, conservation biological control.

A NEW OVIPOSITION-DETECTING ANT CUE PROTECTS MANGOES FROM FRUIT FLIES.

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Background: The mango fruit industry in sub-Saharan Africa suffers quality losses from fruit flies. The impact of African weaver ant (*Oecophylla longinoda*) on fruit fly damage to earlier mango cultivars (cv) was studied, along with the tritrophic interactions between mangoes (*Mangifera indica*), two frugivorous fly species (*Bactrocera invadens* and *Ceratitis cosyra*) and weaver ants. These observations showed that olfactory and visual factors from the presence of *O. longinoda* could help deter these pests. We investigated ant impact on season and late cultivars and the extent to which the density of the pheromone of ants affects the oviposition behaviour of two major mango fruit fly species and resultant damage.

Methods: In the field, we investigated weaver ant impact on three seasonal mango cv (Ifac 3, Améliorée du Cameroun, Kent) and two late cv (Smith and Brooks). We used three blocks in 2008 (Ifac 3, Kent, Smith) and three other blocks in 2009 (Améliorée, Smith and Brooks), each with two treatments repeated 10 times: (i) without ants = control, (ii) with ants. In the lab, the effect of *Oecophylla* cues on *B. invadens* and *C. cosyra* oviposition behaviour were studied using ant-exposed and unexposed mangoes as treatments in 10 replicates. Individual fruits were placed in separate cages and confined with three gravid females of either *B. invadens* or *C. cosyra*.

Results: (1) mean damage inflicted by fruit flies was significantly reduced by 58% and 81% in 2008 and 2009, respectively; (2) tephritid species oviposited significantly less in fruits previously patrolled by ants; (3) for landing, *C. cosyra* seemed twice as sensitive to the ant cues than *B. invadens* on exposed fruits vs. unexposed; (4) although *B. invadens* generally takes longer than *C. cosyra* to oviposit, there was no significant difference between species when confined with ant-exposed fruit; (5) damage from *B. invadens* was six times lower and from *C. cosyra* four times lower for ant-exposed fruits vs. unexposed fruits; (6) *B. invadens* had significantly more pupae per kg of fruit than *C. cosyra* when confined with unexposed mangoes. No significant difference was detected between species when confined with ant-exposed fruits. We discuss the significance of prey recognition of cues from generalist predators and resulting avoidance, together with ecological and behavioural implications.

Conclusions: The use of *Oecophylla* colonies is well-suited to perennial cropping systems in sub-Saharan Africa, as they are efficient, constantly available, widespread and self-regenerating. The effect of cues from generalist predators, such as ants, on the foraging behaviour of frugivorous insects and more generally on pests in terrestrial systems is a relatively new finding likely to have crucial consequences for future research on developing protection strategies against tephritid pests.

Keywords: conservation biological control, predator avoidance, tritrophic relationship, *Mangifera indica*.

REARING THE BIOLOGICAL CONTROL AGENT *DIACHASMIMORPHA KRAUSSI* (FULLAWAY) (HYMENOPTERA: BRACONIDAE) ON IRRADIATED LARVAE OF THE QUEENSLAND FRUIT FLY, *BACTROCERA TRYONI* (FROGGATT) (DIPTERA: TEPHRITIDAE).

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Background: The Queensland fruit fly, *Bactrocera tryoni* is one of the most damaging pests in Australia. Biological control would be a welcome technology. This paper presents results from a study which aimed to assess scope for mass rearing parasitoid wasps on gamma irradiated host larvae. The practical advantage of such a rearing system is that flies and wasps would not need to be separated, thus reducing cost of production. Wasp production could also be done without fear of adverse biological or political consequences arising from escapees.

Methods: *Diachasmimorpha kraussii* adults of both sexes emerged from hosts that were irradiated and exposed as second instar larvae irrespective of dose (0, 4.7, 9.1, 15.9, 27.6, 47.0 and 79.9 Gy).

Results: The fitness of wasps appeared unaffected by the host irradiation; adults from all treatments produced equally numerous offspring. For hosts that were not parasitised, fertile adult *B. tryoni* of both sexes emerged from the control treatment and at the lowest two irradiation doses. Only two flies emerged from the 15.9 Gy treatment and both died within two days. High levels of sterility were not observed for *B. tryoni* at any dose. In similar work in which hosts were irradiated and exposed as third instar larvae, wasps developed regardless of dose. Adult *B. tryoni* of both sexes emerged from the control treatment and the 4.7 and 9.1 Gy doses for all replicates. Emergence declined as the irradiation dose increased and only one fly emerged from the 79.9 Gy dose and this died within a day.

Conclusions: Overall results suggest scope to develop a protocol involving a carefully calibrated irradiation dose and host material of a specific developmental stage that will allow mass production of *D. kraussii*, yet either excludes fly emergence or renders flies sterile from any unparasitised hosts.

Keywords: parasitoid, gamma irradiation, opine, natural enemy

EFFECT OF HOST REARING DIET ON FITNESS PARAMETERS OF *FOPIUS ARISANUS* (SONAN) (BRACONIDAE: HYMENOPTERA).

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Background: *Bactrocera invadens*, an alien invasive pest in Africa poses a significant threat to the livelihoods of millions of African farmers and the entire African economy which is largely agricultural based. To combat the devastating effect of this aggressive pest an IPM package was developed and validated by *icipe*. Classical biological control using a coevolved parasitoid, *Fopius arisanus* (Braconidae: Hymenoptera) constitutes an essential component of this IPM package. However, to insure the releases of this parasitoid on a large scale in all invaded countries, a sustainable and affordable rearing media for mass production of good quality wasps is urgently needed. In this study the effect of three host rearing media; on various fitness parameters of *Fopius arisanus* was evaluated.

Methods: Parasitized *B. invadens* eggs were reared on three media; liquid diet, carrot based diet and mango pulp. Number of recovered puparia, puparia weight, percentage unclosed puparia, percentage of total emerged wasps, developmental time, potential fecundity, adult wasp's size and adult's longevity were recorded.

Results: Total puparia recovery, puparia weight and percentage emerged wasps were significantly higher on liquid diet compared with other diets. Also wasps that emerged from hosts reared on liquid diet had shorter developmental time, larger in size, and their females were more fecund. Wasps' longevity was comparable across the diets. However females lived longer than males in all diet types tested. Unclosed puparia significantly varied with the diet types being lowest when the host was reared on mango but comparable for liquid and carrot diet.

Conclusions: Based on the results of this study, *F. arisanus* wasps from the host reared on the liquid diet were of superior quality for almost all the tested parameters. The potential of adopting the use of liquid diet on a large scale and replacing some of its ingredients with less expensive and readily available local alternatives will be discussed.

Keywords: *Bactrocera invadens*, *Fopius arisanus*, mass rearing, quality control

FIELD SUPERPARASITISM OF *DIACHASMIMORPHA LONGICAUDATA* (HYMENOPTERA: BRACONIDAE) ATTACKING *ANASTREPHA* LARVA ON MANGO FRUITS.

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Background: *Diachasmimorpha longicaudata* is one of the fruit flies larval parasitoids more used as augmentative biological control agent worldwide, given the relative ease of its mass rearing and the high efficiency of its releases in the field. Recent laboratory studies revealed that superparasitism in this species might have adaptive connotations; since it was found a significant correlation between the level of superparasitism and sex ratios biased to females, without detriment in other fitness parameters as fertility and longevity. Our aim in this work was to determine the presence of superparasitism in this species at field level, and study its possible relationship to variables such as host fruit size, size of parasitized larvae, and sex ratio of the emerged adults.

Methods: We collected 498 mango fruits var. Creole (*Manguiфера indica*) in the Valley of Mazapa de Madero, Chiapas, Mexico. We obtained 14,450 *Anastrepha* spp. larvae, which were placed in 1L containers with humid vermiculite to facilitate pupation. Once the pupae were formed, they were measured (length x diameter) with a digital micrometer, and weighed on an analytical balance. The presence of superparasitism was determined by counting the number of scars on each puparium, and sex ratio was determined at the emergence of adult parasitoids (n = 279).

Results: We found that only 37.66% of the larvae were parasitized, and that 22% of them experienced superparasitism. The number of oviposition scars per pupa ranged between 2 and 29 scars, although high levels were rare. The percentage of pupae with more than three scars was 8.11%. Fruit size showed no relationship to levels of parasitism and superparasitism, but it was found that the length and width of the pupae were positively related to superparasitism level ($F_{1, 278} = 16.52$, $P = 0.0001$, $F_{1, 278} = 14.74$, $P = 0.0002$, respectively). Through a logistic regression, we determined that a sex ratio biased to females was positively correlated with the number of scars per pupa and to the length of the pupa, but not with the width (χ^2_1 for number of scars = 11.24, $P = 0.0008$; for pupal length = 4.303, $P = 0.038$; for pupa width = 1.157, $P = 0.21$ NS). The adult emergence and a female biased sex ratio were favored when superparasitism levels were high (> 6 scars / pupa).

Conclusions: Superparasitism is present in natural populations of *D. longicaudata*, and shows advantageous features such as increased adult parasitoid emergence and a female biased sex ratio. These findings are consistent with data found under mass rearing conditions, and support the election of this species as a viable candidate for biological control programs against fruit flies.

Keywords: Parasitoids, Biological control, Fruit flies, sex ratio, oviposition scars.

ASEXUAL VERSUS SEXUAL STRAINS: *WOLBACHIA* INFECTION EFFECTS ON THE BIOLOGICAL CONTROL POTENTIAL OF *ODONTOSEMA ANASTREPHAE* BORGMEIER PARASITOIDS OF FRUIT FLIES.

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Background: The maternally inherited intracellular bacterium, *Wolbachia pipientis*, induces reproductive alterations in host insect populations such as male-killing, feminization, parthenogenesis induction (PI, or reproduction without males) and cytoplasmic incompatibility. Both thelytokous (asexual) and arrhenotokous (sexual) *Odontosema anastrephae* populations attack *Anastrepha* spp. fruit flies on guava in Mexico. Here we report that PI in Mexican *O. anastrephae* populations is caused by *Wolbachia* infection. In addition, we discuss implications derived from laboratory based bionomic comparisons of sexual and asexual *O. anastrephae* strains on their respective mass rearing and fruit fly biological control potentials.

Methods: *Wolbachia* infection in field collected and laboratory reared *O. anastrephae* was detected and sequence typed using PCR. That *Wolbachia* is the likely cause of PI in asexual *O. anastrephae* was determined by PCR screening for alternative bacterial infections (both 16S rDNA general eubacterial primers and primers specific for other bacteria that cause PI) and antibiotic curing (0.5% tetracycline hydrochloride in 10% honey solution). For comparative bionomic studies, treatments of 10 asexual and mated sexual *O. anastrephae* females were each supplied with 10% honey solution as food and equal volumes of late instar *Anastrepha suspensa* larvae in diet as hosts. The experiment incorporated three replications and repetitions and *A. suspensa* larvae controls devoid of parasitoids. The numbers of emergent fruit flies, parasitoids and fruit fly pupae that did not eclose per exposure were counted.

Results: Multi locus sequence typing determined that asexual *O. anastrephae* from Mexico are infected with a novel *Wolbachia* strain (ST 165). That no other bacterial infections were detected and exposure to antibiotics induced males suggests that *Wolbachia* causes PI in Mexican *O. anastrephae* populations. Asexual and sexual *O. anastrephae* sex ratios were 1:64.7 and 1:2.5, respectively, and both strains displayed protandry. The proportion of emergent fruit flies (0.11:0.03) and percentage parasitism (0.33:0.20) in asexual treatments was higher than that in sexual treatments, while a higher proportion of fruit fly pupae demonstrated no eclosion in the latter (0.56:0.77).

Conclusions: *Wolbachia* infection causes PI in *O. anastrephae* producing a higher proportion of females relative to uninfected sexual strains, yet potential infection benefits on mass production of the parasitoid remain unclear. Ongoing experiments aim to further elucidate strain differences and model their relative potential as biological control agents attacking fruit flies on guava in Mexico.

Keywords: Parthenogenesis induction, endosymbionts, MLST, biological control

BACTEROCERA ZONATA (SAUNDERS) STATUS AND ITS NATURAL ENEMIES IN EGYPT.

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Background: The tephritid peach fruit fly (PFF), *Bacterocera zonata* is a recently introduced exotic fruit fly species that has established and become a widespread pest in Egypt. It was ranked as an A1 serious quarantine pest. *B. zonata* threatens countries in the Near East and North Africa and to a lesser extent in Southern Europe. PFF has not established yet in any other region of the Mediterranean or Africa so quarantine concerns are likely to be much more significant for Egypt. PFF poses a threat to many of Egypt's export markets and a more immediate threat to the welfare of the industry than the Medfly and for this reason is likely to prevent further spread. PFF populations are building up slowly from early April reaching high levels in July which persist well in to the fall. Its major host plants in Egypt are; mango, guava, peach, apricot and citrus. PFF has been also recorded as a secondary pest species on some vegetable crops; cucurbits, tomatoes, paprika, eggplant and potatoes in Egypt. Such transaction of the pest from mainly fruit crops to some vegetable crops threatens the production and export of those crops in the future in Egypt.

Methods: PFF detection in Egypt depends on scattered traps using non-toxic methyl eugenol plugs. Control methodology, using killing bags, bait spraying of tree trunks beside chemical control has relatively limited effect on the pest control.

Results: Through a wide survey, natural enemies, particularly native parasitoid species, seem to be absent. For nonnative pests, conservation is likely to be inadequate because sufficiently effective natural enemies will be absent. In such cases, introducing new effective natural enemy species as additional mortality factors against the pest is absolutely essential, and is an approach that historically has been extremely successful. Therefore, an US-Egypt collaborative project was proposed for utilizing the experience of the Plant Protection Dept., in Hawaii, USA in validating and implementing a biological control program for controlling the target fruit flies in Egypt, through testing some exotic specific parasitoid species, as well exchange some of the potential indigenous parasitoid species for the benefit of both sides. Five parasitoid species; *Aganaspis daci*, *Diachasmimorpha kraussi* and *D. tryoni*, *D. longicaudata* and *Fopius arisanus* were introduced from Hawaii for evaluation. The parasitoid species have been maintained under the quarantine conditions for laboratory studies and pilot field trials in Egypt. Preliminary results showed some success of 2-3 parasitoid species.

Conclusions: Establishment of some of those parasitoid species under Egyptian agro-ecosystems will be an additional mortality factor towards the pest control.

Keywords: *Bacterocera zonata*, Status, Natural enemies, Egypt

Session 8

Chemical Ecology, Attractants & Other Control Methods

LETHAL MALE-FEMALE TRANSFER OF INSECTICIDE FROM A FIPRONIL-BASED MALE ANNIHILATION DEVICE BY *BACTROCERA TRYONI* (FROGGATT).

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Background: Male annihilation technique (MAT) devices are an essential component of eradication and crop protection programs for Tephritid fruit flies responding to various parapheromones. In eastern Australia, a mixture of Cue-lure (4-(p-acetoxyphenyl)-2-butanone) and malathion toxicant is commonly used in MAT devices to attract only the male Queensland fruit fly (*B. tryoni*), and on contact, cause rapid death. This paper reports the results of 2 large field cage experiments that demonstrated male *B. tryoni* transferred lethal doses of slow acting fipronil from a new commercial MAT product (Amulet Cue-Lure®) to virgin females during courtship and mating activities.

Methods: In 2 experiments, 50 sexually mature, virgin, laboratory bred male and female *B. tryoni* were released into 3 large (4x4x3 m) field cages, each enclosing a fruiting custard apple (*Anona reticulata* :ANONACEA) tree in a commercial orchard. Release was 1.5 hours prior to their mating time at nightfall and immediately following placement of a single Amulet Cue-lure station in the trees within 2 cages and a non-toxic Cue-lure (control) wick in the 3rd. Mortality of flies was determined in both experiments by collection of cadavers from cages for 4 days post-treatment. In the 2nd experiment an observer in each cage recorded fly behaviour from time of release until dark. At d-1, 14 female cadavers were collected from the Amulet MAT cages and analysed by LC/MS/MS for integument surface residues of fipronil.

Results: Both sexes dispersed into foliage on release. After 2 minutes males aggregated on and around the Cue-lure sources, responding similarly to Amulet MAT and control wicks. Some spent long periods imbibing Cue-lure and regurgitating repeatedly, but on Amulet MATs debilitation of engaged males occurred within 15-30 minutes. Others alighted briefly (1-2 minutes) before flying to adjacent leaves, forming groups of 3-10 individuals, leaf-licking and preening before engaging in aggressive behaviour (head-butting and jostling) to defend a position for stridulation (pheromone release). At dusk, activity increased, females flying to higher leaves and males forming a milling swarm above the tree. Females then moved to leaves near Cue-lure sources where males had stridulated and regurgitated, located a male and copulated. Mating was observed in all cages, but most in the control. Several females alighted on an Amulet MAT. In Amulet cages (Exp.1) male and female mortality at 4-d was 97 & 65% respectively and Cue-lure 28 & 16%. All 14 cadavers analysed (Exp.1 & 2) had fipronil (as total fiprole) residues of 8-1849pg/mg of fly.

Conclusions: The experiments confirmed slow insecticidal activity of fipronil in the Amulet Cue-lure device enabled 2 methods of transmission of lethal residues to female *B. tryoni* to occur: 1. By direct transfer from contaminated male tarsi during copulation, confirmed by analyses; 2. By attraction of females to fipronil contaminated locations on leaves (and Amulet MAT) where males had stridulated and released pheromone.

Keywords: fipronil, Queensland fruit fly, Amulet Cue-lure, MAT.

EFFICACY TRIALS ON EPA K-LIP®-mF, A NEW ATTRACT&KILL DEVICE FOR THE CONTROL OF *CERATITIS CAPITATA* (WIEDEMANN).

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Background: Attract&Kill based control of Medfly has shown to provide excellent results in fruit crop protection. We report here outlined results of efficacy trials on a new device named EPA K-LIP®-mF, which possess noteworthy advantages over existing competitors.

Methods: Devices were provided by manufacturer (Ecología y Protección Agrícola, S.L.). Assays were performed in citrus, stone fruit and persimmon groves in Valencia and Huelva (Spain). Dose effect was studied for each crop. Plot size for each thesis had a surface of approximately one hectare. Damage evaluation was carried on over fruit samples that were kept at evolutionary for 7-10 days before analysis. Female population dynamics was monitorized employing McPhail traps baited with Biolure®.

Results: Suitable dose for citrus is found to be 50 units/Ha. For stone fruits and persimmon the proper dose is standing between 100-150 units/Ha depending on sensitivity of fruit variety. Average fruit damage is below 1% when stated dose are employed. Border effect is clearly observed. These positive results are remarkable due to high levels of infestation in the surroundings attending to damage values of blank plots. Spoiled fruit values for standard chemical treatment plots are clearly higher than those found in EPA K-LIP®-mF treated plots at recommended doses.

Conclusions: EPA K-LIP®-mF has demonstrated to be a helpful tool for protecting fruit crops from *Ceratitis capitata* (Wiedemann) attacks, affording an average damage of less than 1% in small plots with high pest pressure. This method shown to be more efficient than conventional chemical treatments and yields residue-free fruits.

Keywords: Medfly, control, field trials, attract and kill, long-life device.

MONITORING POPULATION DYNAMICS OF ORIENTAL FRUIT FLY WITH WSN TECHNOLOGY.

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Background: Traditional monitoring method on the oriental fruit fly (OFF) population is based on poisoned methyl eugenol trap and labor-intensity counting work. This study adopts wireless sensor network (WSN) technology which combines sensor modules and wireless signal transmission to develop a specialized sensor module to detect the attracted OFFs. With the monitoring system, a sub-hour timescale monitoring database is accomplished, and the wireless signal transmission network allows the distribution of a hectare-wide sensor array to monitor the spatial distribution of the pests.

Methods: The WSN technology was applied to build up an ecological monitoring system for monitoring the OFF population in the Chiayi county, Taiwan since April, 2009. Twenty poisoned methyl eugenol traps were deployed as a 5 × 4 array in an orchard (25 m × 50 m). Each of the traps was coupled with a two-paired infra-red module specially designed for counting the male fly number automatically and equipped a set of sensor node to record the environmental factors as well. The recorded data was then transmitted to a local gateway by wireless network and to the database by Global System of Mobile Communication (GSM) module as a short message (SM) every 30 minutes. The auto-counting data of the traps were individually counted by human twice a week for checking the accuracy of the system.

Results: The year-round population variation just like most surveys, the population climbs high by May, drops in October and keeps low to the following April. However, the hourly population data shows that the male fruit fly is active in the daytime. The earliest trapped individuals are positive correlated to sunrise time, and more individuals are attracted in the morning than afternoon. In terms of spatial distribution, more than 40% of the trapped flies were constantly captured by the most western 4 traps of the orchard through the year. Our results suggest that the OFFs reside in and come directionally from the nearby.

Conclusions: Using the specialized fruit fly sensor module and WSN technology, a nearly real-time monitoring system for the methyl eugenol-attracted male OFF has been accomplished. The hourly data reveals that the fruit flies are attracted in the daytime and mostly in the morning. This auto-counting system provides easier and detailed population data than ever before. Furthermore, grouped sensor array represents the coming direction of local population. Some hot-spots appear in the survey area and the trapped flies of the most western 4 nodes represent more than 40% of the total surveyed number. With the powerful spatial and temporal resolution of population survey provided by the WSN technique, getting insight of the pest population dynamics for future pest control is promising.

Keywords: *Bactrocera dorsalis*, wireless sensor network, population dynamics, methyl eugenol.

EXPLORING STRUCTURE-ACTIVITY RELATIONSHIPS IN THE PHENYLPROPANOIDS TO PROCURE NEW MALE LURES FOR NON-RESPONSIVE *BACTROCERA* AND *DACUS*.

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Background: It is generally thought that between 30 and 50% of *Bactrocera* and *Dacus* spp. do not respond to either of the two recognised male lures, cuelure (CL) and methyl eugenol (ME). Included among these species are pests of quarantine or market access importance, such as *Bactrocera atrisetosa*, *B. cucumis*, *B. decipiens* and *B. jarvisi*. Regulatory authorities are under increasing pressure to report on such pests, making an ability to monitor activity and to use male annihilation as a control/eradication option clearly desirable. While several past studies have looked at structural variants of CL and ME for *B. cucurbitae* and *B. dorsalis* respectively, there has been little research on new male lures for non-responding species in Dacinae endemic areas.

Methods: The study reported here examined 50 different phenylpropanoids (selected on their chemical structures) in a series of trials at a field site in north-east (tropical) Australia, and mostly compared their attractiveness to *Bactrocera* and *Dacus* spp. against raspberry ketone (RK) (the hydrolysed form of CL) and ME. The phenylpropanoids were applied to dental wicks with maldison in 1L modified Steiner traps and left in the field for 6-9 weeks during the wet season.

Results: Ten of the 50 compounds tested did show activity, which varied from weak to very strong attraction. Zingerone (4-hydroxy, 3-methoxyphenyl-2-butanone), which structurally lies between RK and ME, was highly attractive to *B. jarvisi*. It also attracted small numbers of some CL species, the non-lure responding *B. aglaiae*, the rarely caught *Dacus secamoneae* and a totally new species, *B. speewahensis*. The non-lure responding *B. aurea* also came to this compound in a related study in south-east Queensland. Methyl-3-(4-hydroxyphenyl) propionate was the next most powerful lure and attracted a similar range of species (11) to RK (14). It differs structurally from RK in having an additional oxygen molecule in the primary substituent attached to the phenyl ring i.e. methyl propionate instead of 2-butanone. This compound was substantially more attractive than anisylacetone, a precursor of CL. Two-methoxy-4-propyl phenol was the only compound that attracted both *B. jarvisi* and *B. visenda* (a ME species), and the only one that didn't possess a double bond in the primary substituent. Both isoeugenol and methyl isoeugenol are structurally similar to ME but were less attractive to those species that came to the latter. However, isoeugenol attracted a species that is possibly new although similar to *B. kraussi* (a CL species), while methyl isoeugenol attracted *B. nigrovittata*, a non-lure responding species only known previously from New Guinea.

Conclusions: A number of phenylpropanoids have been identified which are attractive to a range of *Bactrocera* that do not respond to either CL or ME and further exploration of their potential through the Asia-Pacific region could prove profitable.

Keywords: Dacinae, phenylpropanoids, new male lures, Australia

IDENTIFICATION OF ROOSTING HOSTS AND THEIR INTEGRATION INTO BAIT APPLICATIONS FOR THE MANAGEMENT OF CUCURBIT FLIES IN WEST AFRICA.

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Background: Several types of protein baits have been used successfully in fruit fly management. In some cases, roosting hosts have been integrated into bait spray programs. Here we report on experiments that identified the most appropriate roosting hosts for cucurbit-infesting fruit flies in Benin (West Africa) and their use as border plants for the spot applications of GF-120.

Methods: Two experiments were conducted at IITA station near Cotonou, Benin. In the first experiment, three main plots (30m x 30m), separated by at least 100m were used. The main plots were planted with zucchini and watermelon. Twelve potential roosting hosts were planted in 5m x 5m plots along the borders - three roosting host plots equally spaced on each side of main plots. Abundance and identity of adult fruit flies were recorded during 10-min visits twice a day (starting at 1200h and at 1800h) at weekly intervals over a period of 2.5 months. Fruit fly infestations of cucurbit fruits were evaluated at two-week intervals. Sorghum and castor bean were selected as most suitable roosting hosts for integration with GF-120 spot sprays in an experiment that followed a design similar to the first experiment, but with six plots – three for GF-120 and three for control. The two roosting hosts were planted in alternating fashion in the four corners of each of the cucurbit plots. Plots were separated by at least 100m. Spot applications of GF-120 at the rate of 2.5 l/ha of cucurbits (1 litre GF-120:1.5 litre water) were carried out at weekly intervals (0700h-0800h) starting at 25 days after planting of cucurbits. Fruit fly monitoring schedules on cucurbits and resting host was similar to the first experiment.

Results: Of the 12 roosting hosts, castor bean was consistently the most suitable for all five species of cucurbit fruit flies encountered in the study site, followed by sorghum; but the latter was most attractive when it was infested with aphids. GF-120 spot sprays were much more attractive to fruit flies on castor bean compared with sorghum. Fruit fly infestations on zucchini and watermelon in GF-120 plots were reduced to 77% and 69% respectively. *Dacus ciliatus* and *D. vertebratus* were the dominant fruit flies infesting zucchini and watermelon respectively. Other fruit flies included *B. cucurbitae*, *D. punctatifrons* and *D. bivittatus*.

Conclusions: GF-120 spot applications on castor bean border plants can be successfully used in integrated management of tephritid fruit flies in cucurbit crops in Benin and likely other countries in West Africa. Large economic returns can be expected from the recommended system because of the high losses in unprotected cucurbits and the efficacy of GF-120 in reducing fruit fly infestations.

Keywords: GF-120, protein bait, *Dacus* spp., *Bactrocera cucurbitae*, integrated management

THE INFLUENCE OF THE PHYSIOLOGICAL AGE OF *CERATITIS CAPITATA* (WIEDEMANN) FEMALES ON THEIR PREFERENCES FOR TWO MASS TRAPPING SYSTEMS.

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Background: Mass trapping techniques have been consolidated as an effective solution to control Medfly *Ceratitidis capitata* (Diptera: Tephritidae) worldwide. One of the reasons for their success is the strong attraction that ammonia odor exerts to the females, which are the damaging fruit agent and the dominant factor for pest multiplication. Among females, it is believed that the youngest are more attracted to proteinic compounds due to the need for an external supply of protein for ovary maturation. With these findings as background, this report further evaluates the influence of the physiological age of the Medfly female on the attraction to two different kinds of mass trapping systems.

Methods: We performed three different crop trials (fig, pome granate and mandarin trees) during Medfly season in the south-east of Spain in order to compare the effectiveness of two mass trapping systems; a solid attractant which contains ammonium acetate, trimethylamine, putrescine and that requires DDVP insecticide (named Standard) and a liquid protein formulation obtained by enzymatic hydrolysis and free of pesticides (Cera Trap[®]). In each trial, system effectiveness was evaluated during three weeks by assessing: (i) the number of captures, (ii) percentage of females and males and (iii) percentage of different physiological females in each system. In order to classify the physiological age of females, we studied their internal reproductive organs through dissection and three categories of ovarium development were established: pre-ovoposition stage, ovoposition stage and senile ovarium.

Results: Total Medfly captures per trap in the three trials performed were significantly higher with the Cera Trap system than with the Standard one. In both systems, the percentage of females was three times larger than males; however, regarding the higher number of total captures of Cera Trap, more females were captured with this system in the three trials. Concerning the physiological age classification, pre-ovoposition and mature flies were highly captured in both Cera Trap and Standard, which implies a significant reduction of the pest pressure for the next generations. The capture of senile flies was considered accidental in both systems. The percentage of total pre-ovopositing females shows a statistical significantly larger amount of captures of these younger flies with the Cera Trap system (60%) compared with the Standard system (40%).

Conclusions: Both mass trapping systems show a great performance on the control of Medfly populations due to the large number of captures per trap during the whole Medfly season, especially for the higher percentage of female flies captured. However, this study shows that Cera Trap system is the most effective, not only for capturing a greater number of Medflies, but especially for its attraction effect on young flies, thus providing control of Medfly population before females are able to damage fruits and multiply the pest population.

Keywords: Medfly, Mass trapping, *Ceratitidis capitata*, physiological age

USE OF LIVE-CAUGHT INSECTS EXPANDS THE SCOPE OF TRAPPING.

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Background: Insect traps are commonly used to draw a sample from wild populations and then use information gained from this sample to infer parameters that apply to the entire population. Insects are almost always killed during the process of trapping, so the datum used is the number of dead flies captured. Extrapolations are then made regarding the actual size of the population and a number of other parameters. Several types of traps, however, can be modified to capture live insects from which we may then derive additional valuable “latent” information on biology and behavior.

Methods: In recent studies [e.g. J. R. Carey et al., *Aging Cell* 7: 426–437 (2008)], *Ceratitis capitata* (Diptera: Tephritidae) were trapped alive with the use of McPhail-type traps baited with a food-based synthetic attractant. The survival patterns of wild-caught individual insects of unknown age were then used to estimate age structure in the populations from which these individuals were derived. Several important findings were obtained from that study. For example, it was found that major changes in age structure occur in wild populations of *C. capitata* and that a substantial fraction of individuals survive to middle age and beyond. The time of capture during the season had a significant effect on the captive lifespan, the average daily reproduction and on the patterns of egg-laying.

Conclusions: The above example provides an illustration of how live-caught insects may be used in trapping as a means of extracting information that cannot be obtained by dead insects. Live-caught insects have a history (related to food, symbiont acquisition, mating, exposure to hardiness, etc) and are loaded with information related not only to aging and reproduction but also to several other parameters in their biology and behavior. Certain of this latent information could be derived in the lab post-capture, thus considerably advancing the scope of sampling science.

Keywords: Trapping, sampling, reproduction, age structure, *Ceratitis capitata*, natural population

EFFICACY ASSESSMENT OF BAIT STATIONS AS A CONTROL METHOD FOR *CERATITIS CAPITATA* (WIEDEMANN) IN FIELD TRIALS.

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Background: Mass trapping or lure and kill methods have been widely used to control fruit fly populations during last century. But the efficacy of these techniques depends on the efficacy of female attractants and the cost and easy to hand properties of the devices. Using high efficacy female attractants the number of required devices can be reduced and, therefore, the replacements of devices that increase the final cost can be prevented. Moreover a well designed device reduces hand labor and increase the killing efficacy. In this work we report the efficacy of new designed bait station devices with more long lasting and efficient female attractants in field trials.

Methods: Two types of bait stations were tested: a chemosterilant one with lufenuron in the bait using 24 traps per ha, and an insecticide bait with cypermethrin applying between 50 and 100 devices per ha. Field trials were conducted in citrus, persimmon and peach orchards and for efficacy assessment fruit fly population and fruit damage before and during harvest were evaluated. Chemosterilant treatment was compared with malathion aerial bait sprays and insecticide baits were compared with terrestrial sprayed insecticides and mass trapping technique.

Results: Chemosterilant devices reduced significantly fruit fly populations regard malathion aerial treatment since the first year of application, but the best results were obtained in the third and fourth year after chemosterilant treatment, when a reduction of 80% of *C. capitata* population was reached. Fruit damage showed a significant reduction in persimmon in the third and fourth year. *C. capitata* populations were also reduced significantly in field with insecticide baits regard insecticide sprayed fields. Fruit damage was maintained below 1% in bait stations treated fields, without significant differences with mass trapping fields. However, in untreated fields, fruit damage was over 25%.

Conclusions: Bait stations were efficient enough to represent an alternative to chemical control with insecticides. In addition, the effect of bait station is long lasting and therefore cumulative effect could be observed if wide areas are treated with this technique. Finally, bait stations add several advantages like non residues in fruit, specificity affectation of target pest and avoid insecticide exposure by applicators.

Keywords: *Ceratitidis capitata*, chemosterilization, bait station, lure and kill.

SPLAT-MAT SPINOSAD ME A NEW CONTROL STRATEGY FOR INVASIVE BACTROCERA SPECIES.

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Background: Tephritid fruit flies include some of the world's most destructive insect pests. Areas infested with fruit flies are quarantined and fruit cannot be shipped to non-infested areas without expensive radiation, cold, or heat treatments that can reduce fruit quality and shelf life. Fruit fly control tactics include the use of cover sprays, toxicant-laced baits, and Male Annihilation Techniques (MAT) that still rely on pyrethroid and organophosphate (OP) insecticides. Public concern over the use of these actives has grown around the world.

Methods: Dow AgroSciences and ISCA Technologies embarked in developing a sprayable novel MAT that would match performance to existing pyrethroid and OP-based options. Spinosad, a green chemistry insecticide, is the toxicant and methyl eugenol (ME) is the attractant. SPLAT, a monolithic passive dispenser, is the basis of the formulation that protects the components from the environment (e.g. rain and UV light) and provides a metered release of the attractant and toxicant, making them available for longer time. This novel product attracts male fruit flies from long distances making it suitable for off-crop applications thus eliminating pesticide and attractant residues on the fruit.

Results: Product was evaluated in Hawaii, USA to assess residual, and rainfastness. SPLAT-MAT™ Spinosad ME and Min-U-Gel-naled MAT were aged for up to 12 weeks with average monthly rainfall of 158 mm during the trial period. Samples were exposed weekly to endemic oriental fruit fly (*Bactrocera dorsalis*) populations. The novel MAT had similar performance to Min-U-Gel-naled MAT during the first 6 weeks and outperformed it from weeks 7-12. In Brazil, two large trials were established to compare one single application of this product to the local MAT standard, wooden blocks dipped in a solution of malathion+ME applied every 20 days, against the carambola fruit fly (*Bactrocera carambolae*). A single application of SPLAT-MAT™ Spinosad ME outperformed multiple applications of the standard treatment during 3 months. In Pakistan a single application of this technology outperformed weekly applications of the standard cover spray, Dipterex, for the peach fruit fly (*Bactrocera zonata*) control in two trials.

Conclusions: SPLAT-MAT™ Spinosad ME, a novel green chemistry-based MAT was developed and is currently registered in Brazil and the USA. It has been tested against three *Bactrocera* species in different geographies. Within each scenario the product outperformed the local standard MAT or cover spray program. Fewer applications were required to regulate male and subsequent female populations while resulting in reduced damaged and infested fruit. Benefits from this technology are: reduction in application costs, elimination of insecticide residues on fruit, increased marketable fruit, and reduction of infested fruit being transported to fruit fly free zones.

Keywords: MAT, Spinosad, oriental fruit fly, peach fruit fly, carambola fruit fly.

ATTRACTION AND FEEDING RESPONSES OF THREE *CERATITIS* SPECIES TO PROTEIN BAITS.

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Background: Recommendations for fruit fly control using baits are usually generic. Insecticidal baits applied as sprays or stations are often assumed to result in the same level of control irrespective of pest fruit fly species. In South Africa, three congeneric species: *Ceratitidis capitata*, *C. rosa* and *C. cosyra* co-exist in the north/north east and eastern areas. The attraction and feeding responses of the three species to proteinaceous baits used in fruit fly control were evaluated in field cages and laboratory bioassays respectively.

Methods: Mature protein-deprived, laboratory-reared *C. capitata*, *C. rosa* and *C. cosyra* were used in all tests. Species were evaluated separately. Attractiveness of HymLure, GF 120 Naturalyte and M3 bait (also known as Questlure and used in the M3 bait station registered for fruit fly control in South Africa) was evaluated in field cages. A novel method of bait evaluation was used where baits tested were each placed in an aerated plastic container which was in turn suspended in an open-ended plastic cylinder lined with a sticky insert to trap attracted flies. Feeding responses of HymLure, Solbait and M3 bait were evaluated separately in the laboratory in aerated plastic containers fitted with a removable glass top. Female flies were released in the containers one hour before bait exposure. All baits were combined with a green food dye at the rate of 1%. Baits were exposed as dried droplets (a drying period of 30 minutes at 30° C) on glass slides which were weighed before exposure to flies. Feeding tests ended after 24 hours when the number of flies containing the green dye was counted and the glass slide containing the bait was weighed to determine the amount of bait consumed per female.

Results: Attraction responses to baits were species-specific. *C. cosyra* had a low response to HymLure but responded well to the other baits. *C. capitata* was the most responsive of all species tested. There was variability in consumption between baits for each species and between species for similar baits.

Conclusions: The study showed differences in responses to protein baits even between closely related congeneric species. As such, in areas of multiple fruit fly pest species, it is essential to choose the appropriate bait or combinations of bait that will work effectively against all species present.

Keywords: *C. capitata*, *C. rosa*, *C. cosyra*, baits

USE OF MAGNET MED TECHNOLOGY FOR THE ATTRACT AND KILL OF MEDITERRANEAN FRUIT FLY (*CERATITIS CAPITATA*).

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Background: The optimization of potent Medfly attractants identified by the USDA has revolutionized the strategies used for the control of this important pest of fruit crops replacing both chemical cover sprays and bait sprays. Till now, however, these attractants have been formulated for use mainly for the mass trapping of Medfly where they applied in combination with suitable traps and killing agents. While very effective these systems are cumbersome and costly. Magnet MED moves this technology on to the next level by combining the attractants; the target device and the killing agent into a single ready to use attract and kill (A&K) system.

Methods: Developed and tested over the last 4 years, Magnet MED offers a highly effective system for the control of Medfly. The presentation will detail the development of the technology and will present the results of trials carried out mainly in Italy and Spain. These demonstrate that A&K gives control equal or better than the current mass trapping systems and is as effective as any conventional insecticide program used for Medfly control.

Results: The system uses very low rates of Deltamethrin insecticide at between 0.5 to 0.75g per hectare for season long control up to 5 months. The system has been optimized so that the application of 50 devices per hectare can now be recommended for most crops with the option to increase to 75 per hectare in vulnerable crops such as peaches. Because the insecticide is not applied directly to the crop and can be removed at the end of the season, the formulation is approved for use in organic orchards in the European Union.

SPINTOR CEBO[®] (GF-120[®]), A UNIQUE CONTROL STRATEGY FOR SUSTAINABLE FRUIT FLY MANAGEMENT IN SPAIN.

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Background: SPINTOR[®] CEBO (GF-120[®], SUCCESS[®], SPINTOR[®] FLY), is a unique pre-mixed fruit fly bait containing spinosad, an organic insecticide, and an optimized blend of feeding attractants. It offers improved efficacy and attractiveness with a favorable environmental and toxicological profile when compared to current toxicant bait mixtures. SPINTOR[®] CEBO attracts and controls males and females of multiple species of tephritid fruit flies. SPINTOR[®] CEBO delivers very low quantities of insecticide per ha (0.24-0.36 grams) decreasing environmental load by 600 to 8,000 times per season when compared to conventional fruit fly control products commonly used in Spain. Spinosad is derived from the fermentation of a soil bacterium, *Saccharopolyspora spinosa*, with a unique mode of action on the insect nervous system. SPINTOR[®] CEBO is currently the only pre-mixed bait authorized in Spain for ground and aerial applications in citrus and olives.

Methods: Field, laboratory research, and commercial applications over the past 10 years in Spain in citrus and olives are summarized. Applications were done using ground or air, equipment customized to achieve optimum spray parameters.

Results: Our experience with SPINTOR[®] CEBO clearly demonstrates that in order to obtain the maximum attraction and residuality the product must be applied as a concentrated bait at rates of 1 – 1.5 L product/ha in 4 to 10 liters of total mixture (with water). The application should deliver droplet sizes of 4-6 mm and should be aimed to the top part of the trees, where adult flies are more active. Ground application equipment was developed to deliver SPINTOR[®] CEBO at the right rates and recommendations resulting in a significant reduction of spray volume (90-95%) versus conventional bait sprays. Since this application method represented a significant change for farmer practices, an extensive training program was implemented. Aerial applications have been adapted resulting in a reduction of spray volume of more than 50%, reducing the total cost and time to treat crops.

Conclusions: Extensive experience on efficacy against *Ceratitis capitata* in citrus and *Bactrocera oleae* in olive has been gathered in the last decade in Spain. When used according to application recommendations, SPINTOR[®] CEBO consistently delivers excellent efficacy for fruit fly control and crop selectivity. SPINTOR[®] CEBO provides key attributes to the growers such as lower application cost, short pre-harvest interval (1 day), EU and USA authorization in Organic farming, favorable environmental and toxicological profile, and compatibility with beneficial arthropods and pollinators. SPINTOR[®] CEBO should be a key component of an integrated fruit fly control strategy for a sustainable agriculture to meet present and future farmer and export market needs.

Keywords: Medfly, olive fruit fly, spinosad bait, organic, aerial application, efficacy

TRITROPHIC INTERACTIONS IN THE COMPLEXES OF FRUIT FLIES DAMAGING FRUIT AND VEGETABLE CROPS IN LA REUNION ISLAND

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Background: The study of tri-trophic interactions is a vast field of basic ecological studies which are also of paramount importance for the development of environment-friendly methods of pest management. Within the Diptera, the Tephritidae family represents an interesting model for such studies because of the variable host-specificity of species in this group, and the important role that some natural enemies may play in the regulation of their populations. Furthermore, the great economic importance of many species in this family offers a large array of applications to research results. La Réunion, a French island situated in the south-west of the Indian Ocean, is a favourable area for studying such interactions, because of the number of tephritid species present in the island, and the great variability of its climatic conditions. On fruit crops, a complex of three polyphagous species of tephritids cause considerable damage: the Mediterranean fruit fly, *Ceratitis capitata*, the Natal fruit fly, *C. rosa*, and the Peach fruit fly, *Bactrocera zonata*. In addition, another complex of Dacini is harmful to cucurbit crops: the Melon fly, *B. cucurbitae*, the Ethiopian cucurbit fly, *Dacus ciliatus*, and the Indian Ocean cucurbit fly, *D. demmerezi*. Conversely, on Solanaceous crops such as tomato, the Tomato fruit fly *Neoceratitis cyanescens*, is the only species of economic importance.

Methods: Over the last twenty years, many field studies allowed us to specify the host-range and relative importance of the different species on cultivated crops. Of particular interest are some host preferences observed in the field in some otherwise very polyphagous species, such as *C. capitata*. Detailed studies (lab, wind tunnel and field cages) were also devoted to the host location behaviour of stenophagous species, taking as a model the tomato fruit fly, *N. cyanescens*. Other behavioural studies also highlighted the preferences of the different species for particular host-plants during foraging or egg-laying behaviour. More recently, studies were focused on the quality of various host or non-host fruits for the pre-imaginal development of the different species, and its influence on their fitness. Though some indigenous parasitoids of the Mediterranean fruit fly have been recorded in the island, most of the natural regulation by parasitoids is due to exotic species imported through classical biological control programmes. This is particularly the case with two species imported from Hawaii, in collaboration with USDA Hawaii and the University of Hawaii: *Psytalia fletcheri*, a larvo-pupal parasitoid of the Melon fly, and, more recently, *Fopius arisanus*, an egg-pupal parasitoid of some *Bactrocera* spp. Following the acclimatization of these two species, field studies allowed us to evaluate their host range, favourite habitats, and impact on host species populations. In addition, laboratory, field-cage and wind tunnel studies improved our knowledge of the stimuli involved in host habitat and host selection behaviours.

Results: Results of these studies are summarized and discussed in relation to the current state of knowledge of insect-plant and host-parasitoid interactions in tephritids, and to their possible applications in pest management.

Keywords: Tephritidae, tri-trophic interactions, insect-plant relationship, host-parasitoid relationship.

Workshop
Longevity, Ageing & SIT

A HIGH-RESOLUTION SYSTEM FOR RECORDING THE DAILY AND LIFETIME BEHAVIORAL AND MOVEMENT PATTERNS OF INDIVIDUAL MEXICAN FRUIT FLIES.

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Background: We developed a system in which the lifetime behavior and movement patterns of the Mexican fruit fly, *Anastrepha ludens*, maintained in 27 cm³ cages is recorded every fifth of a second for one minute three times per hour for 24 hours each day throughout their lives. The data generated from the behavioral monitoring system (BMS) include date, time, 1-of-6 behaviors, and locations in 3-dimensional space (XYZ location). This yields over 12 million data-points for a single fly that lives 100 days. The raw data enable researchers to analyze: (1) behavior including frequency, sequence, age and diel patterns; (2) movement including age and diel patterns, rates, and pathways; and (3) location within the cage by age and time-of-day.

Methods: The hardware of the BMS consists of a pair of cameras, a nine-cage tray to house individual flies in each cage, visible and infrared lights. The cameras are connected to an image processor for short term storage of video images, which are fed to a computer for behavior detection and long-term data storage. The BMS records 3-D coordinates of individual flies and automates classification of six behaviors, termed as resting, moving, walking, flying, feeding and drinking. Two BMS were used to monitor lifetime behaviors of 16 *A. ludens* females with each BMS holding four females on the full diet and four females on sugar only diet. The recording was initiated at the eclosion of the flies and stopped at day 134 when approximately half of the flies were dead.

Results: Flies on both diets spend more than 60% of their time on resting every day over their lifetime. The next most frequent daily behaviors were walking and moving, which, combined, counted approximately 25% of a fly's daily time. Flies on sugar diet had significantly higher walking frequency than those on the full diet. Flying, feeding and drinking occurred at very low frequencies, which counted less than 5% of all the behaviors in most of the days. The frequency of daily walking reached the peak around the age of 20 days and then gradually decreased with age for flies on both diets, suggesting that walking frequency is an age-related parameter. Overall, the frequency of daily flying declined with age after reaching the peak around the age of 20 days, while the frequency of daily feeding and drinking appear to increased with age. During night time between 7 pm when the light is off and 7 am when the light was on, flies spent almost all of their time (>95%) on resting, presumably in sleep, while other behaviors were rare. Walking and flying showed hour-dependent changes and reached the peak period in late afternoon from 3-7 pm. The daily total distance gradually increased in the first 15 days, reached the peak at the age of approximately 20 days and then gradually declined with age for flies on both diets.

Conclusion: This BMS can be easily adopted for a regular research lab. It is the first high resolution machine vision system to have the capacity to record lifetime 3-D positions of an animal and automatically classify lifelong behaviors for months.

Keywords: *Anastrepha ludens*, lifetime behavior, aging, biodemography.

DIFFERENTIAL RESPONSE OF A LONG AND A SHORT-LIVED MEDFLY POPULATION TO LARVAL CROWDING.

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Background: Fruit fly larval competition, within infested fruits, may arise because of increased population densities. This is a form of scramble, intra-specific competition that may affect vital life history traits for immature and adult stages. Older studies have addressed effects of larval crowding on aspects of development and survival of immatures. Nevertheless, little is known regarding the effects of larval crowding on adult life history traits. And, it still remains unknown if different populations (biotypes) selected for short or long adult lifespan exhibit the same response under stressful conditions imposed by larval crowding. Here we investigated the effect of larval crowding on the life-history traits of a long and a short-lived Mediterranean fruit fly, *Ceratitis capitata*, (Wiedemann; Diptera: Tephritidae) populations.

Methods: Experiments were conducted during 2006 – 2007 under constant laboratory conditions (25 ± 1 °C, 65 % R.H., 14:10 L: D). We used a long, and a short-lived medfly population originating from Greece and Guatemala respectively. Newly hatched larvae of the two populations were randomly assigned in six treatments in relation to larval density: 1, 5, 15, 30, 60, and 120 larvae per 3 ml of larval diet. We recorded larval and pupal survival, pupal size (length and weight), and duration of larval and pupal development. Soon after adult emergence pairs consisting of a male and a female from four (1, 15, 60, and 120 larvae per 3 ml of food) out of the six treatments in relation to larval density for both medfly populations used, were placed into cages containing adult diet, water, and oviposition substrates (domes). Mortality and female fecundity were recorded daily until death.

Results & Conclusions: The short and the long – lived medfly populations responded differently to stress induced by larval crowding. Increased larval density (a) reduced larval and pupal survival only in the case of the short-lived population (Guatemala), and (b) increased duration of larval development only for the long-lived population (Greece). Pupal size (length and weight), adult lifespan, and female fecundity were reduced in response to larval density for both populations used.

Keywords: *Ceratitis capitata*, intraspecific competition, stress, life-history, fitness

AGE AFFECTS THE MATING COMPETITIVENESS OF MALE MEDITERRANEAN FRUIT FLIES.

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Background: Mating behavior of male Mediterranean fruit flies *Ceratitis capitata* (Wiedemann) has been extensively studied the last few decades mostly in the frameworks of the implementation of the Sterile Insect Technique (SIT). However, the effect of age of males on mating success remains largely unknown, especially for the older males. When sperm resources are not exhausted in successive matings, theory predicts that the older males would be more successful against young males in receiving matings. We used medfly males, a male lekking species, to test the above hypothesis.

Methods: Experiments were conducted during 2007 – 2008 under laboratory conditions. Wild-like flies, reared in laboratory for 1 – 3 generations were used. We run mating tests under competitive (two choice) and non competitive (no choice) conditions. In choice tests, 10 young (12-14 days old), sexually mature, virgin females were brought to choose between 10 control (sexually mature, non mated, young, 12-14 days old) and 10 older males (15, 20, 30, 40, 50 days old). In no choice tests, the performance of 10 non mated males of different age (10, 15, 20, 30, 40, 50 and 70 days old) to mate with 10 young, sexually mature virgin females was assessed. In choice tests we recorded the number of copulas of young (control) and older males. In no choice tests we further recorded the copula duration, the latency time, the amount of sperm transferred to female spermathecae, and the sperm abundance in testes of mated, and not mated males.

Results: In choice tests male mating competitiveness dramatically decreased in ages older than 30 days. Fifty days old males achieved only 15% of matings compared to younger – control males. The proportion of matings decreased in older ages in no choice tests. Nevertheless, more than 30 and 20% of 50 and 70 days old males achieved copulations respectively. Mean duration of copula decreased in older males while the latency time to mate increased. Old males transferred to female spermathecae comparable amounts of spermatozooids with that of younger males. The number of sperm cells increased in testes of both mated and non mated males at older ages.

Conclusions: Our results indicate that (I) *C. capitata* male mating competitiveness decreases in advanced ages (II) although ageing negatively affects the ability of males to mate (even in no competitive conditions), older males can still become accepted as mating partners, transferring comparable amounts of spermatozooids with that of younger males to female spermathecae. And (III) our data suggest that spermatogenesis occurs in adult males until extreme ages. The importance of our findings to understand the role of older males for the reproductive behavior of male lekking species is discussed.

Keywords: Medfly, aging, mating competitiveness, sperm transfer

POSTER ABSTRACTS

DATABASES

RIESPO: IBERIAN NETWORK ON THE EVALUATION OF EFFICACY AND SIDE EFFECTS OF CONTROL TREATMENTS AGAINST OLIVE PESTS

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The Iberian network on efficacy and side effects evaluation of control treatments against olive pests (RIESPO) was constituted in 2009 and funded by a Complementary Action (AC2009-00045-00-00) of the National Institute of Agricultural and Food Research and Technology (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, INIA). The main objectives of this network is unifying and standardize methodologies applied in field trials, from the setting up of the assays in the field to the sampling methods used to characterize the arthropod community of olive groves and the statistical methods used for data analysis. Currently, the network consists of researchers and technicians from eight centres in Spain and Portugal. Members participate in research projects focused on pest control in olive groves, paying special attention to the key pest of this crop, the olive fruit fly (*Bactrocera oleae*). This communication aims to present the network RIESPO to those researchers who may be interested in its activities.

Keywords: *Bactrocera oleae*, Field trials, Control methods, Efficacy and side effects assessment

NEW PROGRESS IN TEPHRITID WORKERS NETWORKING

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Tephritid Workers Database (TWD) was established in 2004, and since then the number of its member keeps growing to reach 1046. To ensure excellence and relevance in networking among tephritid workers, TWD intends to evolve to achieve this mission. In 2009, TWD knew two major developments, the establishment of the Tephritid Workers of Western Hemisphere Database (TWWH) and the reinstatement of the Fruit Fly News (FFN) newsletters.

It took almost one year of hard work to establish the TWWH since the decision was taken at the 7th Meeting of the Working Group on Fruit Flies of the Western Hemisphere in Mazatlan, Mexico on November 2008. At present, TWWH is the largest regional group, including 425 of tephritid workers from 21 countries. The TWWH database is bringing together scientists from major universities, research institutes and agriculture agencies in the Americas. Their field of expertise is extending from ecological theory and laboratory investigation to area-wide programs using the latest technology to control fruit flies.

Fruit Fly News (FFN) was initially produced in hardcopy form in the 1980's, and used to be the communication tool of the IOBC Global Working Group on Fruit Flies of Economic Importance. A total of 11 annual or semiannual issues were produced until 1992. Now, 17 years later, FFN is back and is provided online from the issue FFN 12. The goal of FFN is to become the communication tool of the worldwide groups working on tephritid fruit flies, providing timely information on management of tephritid fruit flies, research activities, publications, ongoing action programs, upcoming events, job opportunities, and news. As a community means of communication, efforts are made to ensure its sustainability and regular frequency of delivery. Up to now, there are three issues a year. All contributions and suggestions are welcome and highly appreciated.

Keywords: Database, newsletter, tephritid, fruit flies, pest

Session 1
Biology, Ecology & Behaviour

SEXUAL COMPATIBILITY AMONG POPULATIONS OF *ANASTREPHA FRATERCULUS* (WIEDEMANN, 1981) (DIPTERA: TEPHRITIDAE) OF DIFFERENT REGIONS OF PERU.

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Background: An essential requirement for the application of the Sterile Insect Technique (SIT) is the evaluation of sexual behavior of wild flies to predict the expected performance of laboratory colonies under field conditions, the aim of this study was evaluated the sexual behavior of mass-reared flies and wild populations of *Anastrepha fraterculus* from six regions of Peru where the SIT could be applied.

Methods: We evaluate the sexual compatibility among populations of the Peruvian Coast: Piura, Lambayeque, La Libertad, Ancash, Lima, Ica and mass-reared flies, six field cages installed on plants of *Persea americana* Mill were used; the wild flies were 16 days old and laboratory flies 9 days old. 30 females and 30 males from each population (two at a time) were released in each cage, in four replicates and couples from a single population as witnesses were released in the other ones (two cages). After each test the crosses: females of the 2nd population with males of the 1st population and females of the 1st population with male of the 2nd population were carried to laboratory and reproduce them in host fruits.

Results: The results showed that populations were sexually active showing more activity around noon with some differences in the starting time, 9:00 and 10:00 am and peaks of sexual activity at 11:00, 12:00 and 13:00. Nevertheless the index of sexual isolation (ISI) ranging from -0.07 to 0.28, reflecting sexual compatibility between the populations and the mass-reared strain, indicating that wild individuals successfully mate with each other and with the laboratory strain. Also the male relative performance index (MRPI) and insect females (FRPI) ranging from -0.26 to 0.36 reflecting both sexes of insects produced in the laboratory is as effective as the wild to mate.

Conclusions: Our results of behavior, it is confirmed that the colony used for mass rearing of sterile insects for the implementation of sterile insect technique for pest control is compatible with the flies from the regions studied. Efforts should be initiated to determine if populations of other natural mountain and forest regions in the same group and the feasibility of applying the sterile insect in these regions.

Keywords: *Anastrepha fraterculus*, Tephritidae, SIT, sexual compatibility, Peru.

SUSCEPTIBILITY OF *SECHIAM EDULE* TO FRUIT FLIES (DIPTERA: TEPHRITIDAE) IN LABORATORY CONDITIONS

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Background: *Sechium edule* (chayote) is a climbing cucurbit largely cultivated under arbour in La Réunion but its production has strongly declined in recent years. Farmers attribute the yield losses to fruit flies (Tephritidae, Dacini) (*Bactrocera cucurbitae*, *Dacus ciliatus* and *Dacus demmerezi*), accordingly current studies are carried out in order to evaluate in the field the damage of fruit flies. Our study aimed to assess in laboratory the susceptibility of *S. edule* to fruit flies (*B. cucurbitae*, *D. ciliatus*, *D. demmerezi*), particularly in studying (i) the future of eggs laid naturally in fruit by gravid females, and (ii) the development of larvae artificially deposited in fruit.

Methods: Two types of experiments were conducted in 2009 and three types of fruit were compared: *S. edule* (young fruit <10cm and mature fruit >10cm) and *C. pepo* as a control. In the first trial, for each species of flies, fruit (*S. edule* and *C. pepo*) were individually exposed to cohorts including gravid females and the number of pupae obtained and adult flies emerged from each fruit were then counted after two weeks. In a second trial, pieces of fruit were artificially infested by L1 larvae of each species, and their survival rate was measured over six days.

Results: Development of the three species was better in *C. pepo* than in *S. edule*, for *B. cucurbitae* (a mean of 615 pupae per fruit of *C. pepo* versus 0 pupae in *S. edule*), for *D. demmerezi* (445 versus 250 pupae), and for *D. ciliatus* (15 versus 10 pupae). The mean number of *B. cucurbitae* pupae obtained per fruit of *C. pepo* was not significantly different ($P>0.05$) from that of *D. demmerezi*. Complete development (eggs to adult flies) on *S. edule* was observed only for *D. demmerezi* and *D. ciliatus*. However, *D. demmerezi* showed better larval survival and emergence rate on *S. edule* than *D. ciliatus*. For both species, larval development was better in younger fruits than in mature ones.

Conclusions: These results provide new information on the development of fruit flies in *S. edule*. The low levels of infestation observed and the difficulties of development for the larvae suggest that fruit flies might not be the only cause of yield losses in chayote crops. These results are important to provide guidelines and to adapt the fruit flies management scheme to the particular case of *S. edule* crops.

Keywords: *Sechium edule*, fruit flies, larval development, emergence rate, pupae

HOW DO ALTERNATIVE HOST FRUIT TREES INCREASE *CERATITIS CAPITATA* (WIEDEMANN) POPULATIONS IN CITRUS CLEMENTINE GROVES?

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Background: *Ceratitis capitata* is a polyphagous specie that causes great losses in citrus crops. The presence of alternative host fruit trees has been proved to lead the Medfly dynamics in citrus groves. Frequently, home gardens and also isolated host trees are close to early Clementine varieties that ripen during the same period. Thus, sanitary measures are promoted to prevent medfly populations to migrate to citrus orchards.

Methods: During 4 years the influence of alternative host fruit trees was studied in Clementine citrus orchards. From 6 to 7 Tephri-Trap lured with Tri-Pack, plus DDVP as a toxicant were placed along the citrus orchard. The number of adults caught per trap was recorded weekly. Fruit maturity was determined by measuring the rind colour index with a Minolta CR-400® chromameter. In addition, during 2 years, 274 samples of 14 different fruit species were collected weekly from all the citrus growing area and placed in containers at 25° C where medfly larvae emerged were counted. Fruits were counted and weighted, thus the number of larvae per fruit and kg of fruit was quantified.

Results: All the sampled fruit species, both cultivated and wild were attacked by medfly. Figs and jujube fruits produced the highest amount of medfly larvae, over 100 larvae per kilo. The sanitary measures on stone fruit orchards (basically peaches) surrounding citrus had a strong influence on medfly populations, but did not threaten the harvest since did not coincide with citrus ripening. Medfly adults from overripe fruits in orchard borders moved and achieved distances as shown by captures in traps. Medfly control by mass-trapping in those fruit trees was not enough to prevent the migration of insects to the citrus crop, since captures on the border of the orchard were high.

Conclusions: Both fig and jujube trees that grow in the nearness of citrus crops, produce the highest medfly populations when early Clementine varieties are susceptible to be attacked by this pests. Consequently, enhancing mass trapping technique in areas is required even when pest control in alternative host fruit trees is performed.

Keywords: Medfly, *Ceratitis capitata*, Clementine, alternative host trees, traps.

PERFORMANCE OF AUGMENTORIUM AS A SANITATION TECHNIQUE AGAINST FRUIT FLIES (DIPTERA: TEPHRITIDAE) IN REUNION ISLAND

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Background. Tephritid fruit flies cause severe damage to fruit and vegetable crops in Reunion Island. Instead of the curative approach to reduce existing populations, the first step proposed for their management is sanitation. This method is based on an original technique firstly developed by USDA in Hawaii utilizing a tent-like structure called an “augmentorium” which aims to sequester adult flies emerging from infested fruit while allowing the parasitoids to escape, via a net placed at the top of the structure. This study focused on the performance and the efficiency of the augmentorium prototype recently tested in Reunion Island and particularly (i) the number of adult flies that can potentially be sequestered in an augmentorium in the field; (ii) the efficiency of the net mesh for fly sequestration and parasitoid escape; (iii) the feasibility of producing compost with infested fruit collected in the field.

Method. The potential number of flies that could be sequestered was estimated by measuring in the lab the emergence of several species of flies from infested fruit collected in the field from 2005 to 2009 in different sites of the island. Emergence of adult flies was measured for six species of flies: (i) *Bactrocera cucurbitae*, *Dacus ciliatus* and *D. demmerezi* attacking three species of Cucurbits (pumpkin: *Cucurbita maxima*; cucumber: *Cucumis sativus* and courgette: *Cucurbita pepo*); (ii) *Bactrocera zonata*, *Ceratitis rosa* and *C. capitata* attacking one species of fruit (mango: *Mangifera indica*). The sequestration of three of these fly species (*B. cucurbitae*, *B. zonata* and *C. capitata*) and the escape of two of their parasitoids (*Psytalia fletcheri* and *Fopius arisanus*) were assessed in the Cirad laboratory in Saint-Pierre in 2008. Preliminary tests on the feasibility of producing compost were then conducted in Saint-Pierre in 2009, mixing courgette and other components.

Results. Collections of infested fruits showed the following means of emerged adults per kg of fruit: 76 for mango (*B. zonata*, *C. rosa*, *C. capitata*); 217 for cucumber, 340 for pumpkin and 594 for courgette (*B. cucurbitae*, *D. ciliatus*, *D. demmerezi*). The efficiency of the mesh chosen for the prototype of augmentorium (hole area 1.96 mm²) proved to be perfectly effective in the lab with 100% of sequestration of adult flies. In the same way, 100% of the parasitoids were able to escape from the mesh if they choose to do so. In addition, we showed that a ratio of 50:30:20 of courgette, sugar cane stem and chicken litter respectively was well adapted to produce compost.

Conclusion. These results confirm the relevance and the efficiency of the augmentorium in an agroecological crop protection. As a sanitation technique against fruit flies, the augmentorium sequesters on average several hundreds of adult flies per kg of infested fruit. As a biological control method, it may contribute to increase parasitoid populations which are often low because of the previous and significant pesticide pressure. The augmentorium can also be considered as a useful tool to produce compost in the context of sustainable agriculture. The technique of sanitation using the augmentorium is now well accepted by farmers in pilot areas in Reunion Island.

IMPACT OF FRUIT FLIES (DIPTERA: TEPHRITIDAE) ON CHAYOTE (*SECHIAM EDULE*) IN REUNION ISLAND.

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Background. Chayote is a popular vegetable in Reunion Island. Traditionally and extensively cultivated for its leaves, it is nowadays usually intensively cultivated under trellises for the production of fruit. A high number of fruit fallen on the ground result in a significant loss in production. Farmers attribute this to three fruit fly species (*Bactrocera cucurbitae*, *Dacus ciliatus* and *Dacus demmerezi*) which roost in the trellises. This situation provokes major insecticide applications in the chayote trellises to combat fruit flies. The present study aimed therefore at evaluating the real incidence of fruit flies in chayote cultivation.

Methods. The experiments were conducted in 2008 and 2009 and observations in trellised crops were performed in different sites on the island. In 2008, hundreds of fruit were collected at a single site to study the susceptible stage of the chayote fruit while the development of samples of 20 recently blemished fruit and 20 unblemished fruit was regularly followed in the field. In addition, 120 blemished fruit were collected in 2008 in two sites 20 fruit were weekly collected at a site in 2009 and the emergence of adult flies was measured in the Cirad laboratory over 15 days. In addition, bird nets were placed under the trellis of three fields in 2009 to collect fallen fruit and the number of blemishes and the infestation of the fruit gathered were examined. In the lab, eight chayote fruit were exposed to gravid females per fly species and dissected.

Results. *In situ*, fruit smaller than 6 cm length appeared to be unaffected by female blemishes. Two to four weeks after blemishes under the trellis, the blemished fruit did not show any morphological difference compared to unblemished fruit. This observation was strengthened by the results of emergence tests: only one of 120 blemished fruit collected in 2008 and only five fruit collected from 219 fruit harvested in the crop in 2009 showed emergence of flies. *D. ciliatus* appeared to be the only species able to emerge not only from these infested fruit collected in the field but also from the fruit exposed to gravid females in the lab. A defence reaction of the fruit to eggs or L1 larvae of *B. cucurbitae* and *D. demmerezi* was also observed by dissecting infested fruit in the lab. Finally, other field observations showed that only 13 of 197 fruit fallen from the trellis were infested by larvae, meaning that 93 % of fruit fell for other reasons.

Conclusion. This study provides first results on the real incidence of fruit flies in chayote crops in Reunion Island. Although fly adults roost in chayote trellis, these pests are not responsible for the significant production losses recorded by the farmers. This suggests that the chemical protection used against fruit flies in chayote trellis is not appropriate, especially when one considers the secondary negative effects of the chemical treatments. A physiological shedding caused by an overloaded production of fruit under this type of intensive cultivation under trellis could be the main cause of the fruit fall and this requires further research.

Keywords: fruit flies, chayote, *Sechium edule*, Reunion Island

INFERENCES ON COMPETITIVE INTERACTIONS FROM DISTRIBUTION PATTERNS OF COEXISTING *ANASTREPHA FRATERCULUS* AND *CERATITIS CAPITATA* POPULATIONS.

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Background: *Anastrepha fraterculus* (native to South America) and *Ceratitis capitata* (exotic species in this region) have shared host fruit species in Argentina for more than one century. Little is known about the mechanisms that allow the coexistence of these tephritids. Previous works suggested that avoidance of infested fruit would act as a stabilizing force minimizing the negative effects of interspecific competition. We tested here the hypothesis that these species exploit available resources avoiding larval competition by analyzing the spatial distribution of infestation, particularly co-occurrence patterns.

Methods: *A. fraterculus* and *C. capitata* infestation patterns were evaluated from pupae obtained from *Psidium guajava*. Fruit were collected in Northeast region of Argentina (Concordia) and were processed individually. Frequencies of fruit infested by one, both or none species were compared with two theoretical distributions, expected under a random oviposition hypothesis and under an avoidance (of already infested fruits) hypothesis. As an alternative method we use null models (Ecosim 7.0). Each method was tested at three spatial scales, for each sampling date.

Results: Both methods showed that infestation patterns varied according to the spatial scale. At the largest scale there was a tendency towards co-infestation, while at a reduced scale (particularly at the tree scale), the pattern revealed either a random oviposition behavior or avoidance of infested fruit. At this scale, a tendency towards avoiding infested fruit occurred only when fruit infestation levels were moderate.

Conclusions: Our study suggests that competition avoidance behavior could be density-dependent. This mechanism could, at least partially, permit the coexistence of *A. fraterculus* and *C. capitata*. Other factors, such as environments and host species associated with agroecosystems could also be playing a role in structuring population densities.

Keywords: Interspecific competition, distribution patterns, EcoSim, spatial and temporal heterogeneity.

LIFE CYCLE ASSAY AND ESTIMATING SURVIVAL FUNCTIONS OF KONAR FRUIT FLY, *CARPOMYA VESUVIANA* COSTAL IN SOUTH OF IRAN.

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Background: The Konar fruit fly, *Carpomya vesuviana* Costa (Diptera: Tephritidae) cause high damage in quality and quantity of (Konar) *Ziziphus* spp. in south of Iran. The life table is one of the most important tools in demographic and gerontology research because it is used to characterize the mortality and survival properties of cohorts and to quantify the actuarial rate of aging.

Methods: Field and laboratory studies on the Life cycle of *Carpomya vesuviana* were carried out for the three consecutive years in south of Iran. The materials of the Tephritid (Eggs, Larva, Prepupa and Pupa) were collected by weekly sampling in nature and reared in Petri dishes containing the soft well aerated soil. The Konar fruit fly was reared on three kinds of *Ziziphus* fruits (*Z. spina-christi*, *Z. nummularia*, *Z. mauritiana*) as well as an artificial diet to study the life cycle parameters under laboratory conditions.

Results: The longevity of adults was from 14.6±4.2 to 25.8±6.3 days in different months. The sex ratio was 1: 1. The pre-oviposition, oviposition and post-oviposition periods lasted 2 to 8, 3 to 35 and 0 to 12 days, respectively. The female lays egg after mating under fruit skin. The number of eggs/ovipunctures observed in a single fruit is generally 1 to 4 and a maximum of 8. Females laid averagely 19.1±5 eggs. Eggs-laying in most females (about 72.8%) is carried out in the first 3 to 7 days of their life. Females have higher fecundity during November and February and lower fecundity in March. The incubation period was 1-4 days and about 70.4 to 91.9 percent eggs turned out to be viable. The larval period was 6 to 22 days. Larvae had longer during December (15.5±3.6) and lived short during March (average 8.5±1.6). The maggots took 1.8 to 5 hours to prepare puparium. Pupal duration was variably different between 8-320 days. The shortest was 8 days in March April and the longest was 320 days in September.

Conclusions: The insect completed 8 to 10 overlapping generations each year. The shortest time for a complete cycle belonged to the flies which laid eggs in March, April and January. The longest time for a complete cycle was about 330 day which refers to the eggs laid in September-October. The over wintering and summering was a pupa within soil profile. This insect has diapause or might not have any diapause at all. Insect attack intensity was estimated between 30 and 100 percent in randomly inspected fruits.

Keywords: *Carpomya vesuviana*, Konar, *Ziziphus*, Life cycle, Iran

MATING COMPETITIVENESS OF STERILIZED MASS-REARED MALES AND WILD POPULATIONS OF *ANASTREPHA FRATERCULUS* (WIEDEMANN, 1981) (DIPTERA: TEPHRITIDAE).

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Background: The South American fruit fly *Anastrepha fraterculus* (Wiedemann 1830), is one of the most important agriculture pest in Peru. The Sterile Insect Technique (SIT) would be a powerful no-pollutant method for its direct control which has been used successfully against a parental specie *Ceratitis capitata* (Wiedemann 1829) in Peru. The success of SIT depends on the quality and the ability of sterile males mate with wild females. Competitiveness tests measure the ability of sterile males to achieve copulations with wild females and the degree of the sterility of the eggs produced by wild females when wild and sterile males compete to mate with them. The aim of this study was to evaluate the mating ability of sterilized mass-reared (two doses 0.07 and 0.08 KGy/hour) *A. fraterculus* in comparison with wild males from 5 regions of the Peruvian coast: Lima, Ica, Ancash, La Libertad and Lambayeque.

Methods: “competitiveness” cage (field cage) into which 120 insects sterile males were release along 40 wild males and 40 wild females; “wild control” cage (plexiglas cage 30 cm×30 cm×40cm) into which 25 males insects wild were release along with 25 wild females; “sterile control” cage (cages with panel 20x15x15cm) into which 25 sterile males were released along with 25 females fertile insect. Plums were placed into each cage for a period of 8 days in order to collect the eggs to measure the induced sterility. The fruits hosts were changed daily and the percent of eggs hatched was evaluated. Fried's competitiveness index (C) was used whose values range from 1 to 0, values close to zero indicate superior competitiveness of the wild male. The weight of the pupae were registered in order to correlate the average weight of the irradiated pupae and the C value

Results: The results obtained in the Fried test (Which measures induced sterility in eggs) with irradiated pupae with 0.08 KGy/hour showed a competitiveness coefficient ranging from 0.27 to 0.47; on the other hand irradiated pupae with 0.07 KGy/hour showed a competitiveness coefficient ranging from 0.30 to 0.45. The lowest sexual performance of the sterile males was with the Ica population (in both doses), while the highest ones was with the La Libertad population (0.08 KGy/hour) and Ancash (0.07 KGy/hour); this suggests that sterile males successfully compete with flies from different parts of the Peruvian coast.

Conclusions: Mating competitiveness is an important quality control parameter that affects the performance of released sterile insects. The results suggest that sterile males successfully compete with flies from different parts of the Peruvian coast. This paper shows that despite the strain of mass rearing *A. fraterculus* used in the test has been reared since 2001 is still appropriate for the purposes of implementing the SIT.

Keywords: *Anastrepha fraterculus*, SIT, sexual Competitiveness, sterile male, wild flies.

DISPERSAL DYNAMICS OF AN INVASIVE FRUIT FLY SPECIES, *BACTROCERA INVADENS*, ALONG AN ALTITUDINAL GRADIENT.

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Background: *Bactrocera invadens*, a fruit fly species originating from Asia, has become an invasive pest in Africa. This fruit fly causes a lot of damage to crops and threatens fruit export, leading to considerable economic losses. In Africa it seems to be spreading not only in latitude but also in altitude. The ongoing spread of *B. invadens* can be limited by certain barriers: climatic conditions, host availability and suitability, and interspecific competition with cold-tolerant, *Ceratitis rosa*, a native species.

Methods: This study tried to determine the boundaries for the extension of the distribution area of *B. invadens* by placing traps and collecting fruit along an altitudinal transect (580-1789 m) in Morogoro, Tanzania from September 2008 until September 2009. Concurrently environmental variables (temperature, relative humidity and host availability) were measured along the transect at the different collection sites.

Results: Diversity increased with rising altitude and was determined by host availability and suitability. *B. invadens* abundance and infestation declined with rising altitude and was largely determined by temperature and host availability. Interspecific competition could also play a part in determining abundance and diversity. In peach *B. invadens* was dominated in numbers by *C. rosa*, but in mango *B. invadens* dominated all other fruit fly species, even mango specialist: *C. cosyra*. There was an annual dispersal (in January and February) of *B. invadens* towards higher altitudes in periods with population surplus. Its reproduction in high altitude fruits such as peach and pear could mean a colonization of this area.

Conclusions: Because of *B. invadens*' strong r- and K-selection and its broad climatic and host range it is possible that this species will continue to spread to high altitude areas and possibly pose a threat to subtropical areas, even more so considering the influence of climate change.

Keywords: *B. invadens*, altitude, dispersal, Africa

TEPHRITIDS IN THE MAIN CITRUS-GROWING AREA IN COSTA RICA (2007 – 2008).

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Background: In Costa Rica, 27,000 hectares of land currently produce citrus fruits, these being mainly sweet oranges (*Citrus sinensis*). There are 4,519,219 trees for monoculture crops planted in a total of 20,000 hectares. 15,153 hectares are located in the Huetar Zone, which occupies a significant part of the northern sector of the country. This area is where most of the nation's orange crop is raised. These plantations are located on the border with Nicaragua, and they extend into that country's territory, making oranges the most important crop in that region. In this same zone, the Tico Frut Company operates its plants and other facilities. Tico Frut is Central America's largest industrial fruit processing enterprise, and the most important fruit harvest collection and storage center in all of Costa Rica. In order to best ascertain how to select the most effective and suitable strategies for tephritid control, the diverse tephritid populations present in the aforementioned region were studied.

Methods: The study was carried out at six orange plantations (Farms No. 9, 12, 13A, Naranya, Mefisa, and LBA), these having a total joint area of 5,604 hectares, during the harvest periods of 2007 - 2008. Yellow Multilure Traps that had been baited with Nu Lure were used, with checks carried out each week in order to collect and identify the insects that were captured. Simultaneously, a weekly sampling of 60 ripe oranges took place in the same lots where the traps had been placed. Larvae and pupae were collected on a weekly basis, as their eclosion was expected, which would make it possible to identify them.

Results: A total of 1093 traps were set at the plantations; in these traps, 9,009 diptera specimens were collected, of which 69 were Tephritidae, 803 were Lonchaeidae, and 907 were Ulididae. The number of tephritid orange pests was low. There were only 2 Mexican fruit flies (*Anastrepha ludens*) and 2 Medflies (*Ceratitis capitata*). Other potential orange pest specimens of the *Anastrepha* genus were captured. These were 14 *A. striata*, 33 *A. obliqua*, 5 *A. serpentina* and, 2 *A. fraterculus*. None of these specimens were trapped in or on fruits, but rather only in traps. In the orange crop sampling, 22,920 fruits were analyzed (382 total samples comprised of 60 oranges each) and from these samples, the following pests were obtained: 1,154 Lonchaeidae, as well as 380 Muscidae, but no Tephritidae or Ulididae flies. An unforeseen outcome was that the Lonchaeidae *Neosilba batesi* caught in traps and in the fruit sampling accounted for the majority of the pests collected. Another unexpected result was the number of the Ulididae *Xanthacrona bipustulata* captured in traps. The largest number and widest diversity of tephritids were captured in the area of the industrial plant facilities. In 2007, 112 traps were set, and 1,360 diptera were collected, of which 152 were tephritids (6 *A. ludens*, 1 *C. capitata*, 116 *A. striata*, 18 *A. obliqua*, and 4 *A. fraterculus*), in addition to 85 lonchaeids. In 2008, 75 traps were set, and these caught 383 flies, of which 28 were tephritids (2 *A. ludens*, 2 *C. capitata*, 18 *A. striata*, and 6 *A. obliqua*), but no lonchaeids. This greater diversity was due to the large quantity of fruit stored at this locale, originating from crops harvested at a number of sites around the country.

Keywords: Medfly, Mexican Fruit Fly, *Anastrepha* spp., Lonchaeidae, Ulididae.

DYNAMIC POPULATION OF FRUIT FLIES IN CASSAVA CROPS (*Manihot esculenta*)

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Background: Cassava is a subsistence crop, especially for small proprietiers in the north and NE regions of Brazil. Many insect pests infest cassava crops in Brazil. The damage increases according to technological level and its intensive production. Tephritidae larvae infest easily fruits and stems. The purpose of this work was to determine the dynamic population and identify de fruit fly diversity in a mixed cassava cultivars. The cassava crops is situated in Presidente Prudente municipality, State of São Paulo, Brazil (UTM 7545288.76 m N, 459930.31 m E and altitude 424.29 m).

Methods: The experiment was conducted from February to May 2005, January 2006 to December 2007 in cassava field composed by industry and table cultivars 'IAC-12', 'IAC-13', 'IAC-14', 'IAC-15', 'Espeto', 'Branca de Santa Catarina', 'Roxinha', 'Fécua Branca', 'Fibra' and 'IAC 576-70'. The flies were weekly collected by two yellow McPhail plastic traps at 1.20m high, using 400 mL per trap of 5% hydrolisated protein. Mature cassava fruits were picked at random for evaluation of fruit fly species incidence. The area was kept free of insecticide sprays.

Results: We captured a total of 600 flies from the genus *Anastrepha* (372 males and 228 females), related to: *A. montei* Lima (152), *A. sororcula* Zucchi (6), *A. pickeli* Lima (22) and *A. fraterculus* Wied. (2). Based on the flies per trap per day (FTD), the peak reached 1.64 (13/4/2005), 1.43 (12/04 and 17/05/2006) and 2.21 (18/04/2007). The infestation rate in fruits was 2.0% in 'Espeto' and 'IAC 14'. Only *A. montei* was recovered from cassava fruits.

Conclusions: Low fruit fly diversity was registered in cassava crops by McPhail trapping, which only *A. pickeli* and *A. montei* was related with cassava plants. We obtained low population in cassava fruits by *A. montei*.

Keywords: McPhail traps, FTD, *Anastrepha*, occurrence.

OCCURRENCE OF FRUIT FLIES IN MANGO ORCHARD IN THE WEST REGION OF THE STATE OF SÃO PAULO, BRAZIL.

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Background: The mango crops are widely distributed in the majority of tropical and subtropical countries. Fruit flies (Diptera: Tephritidae) are key pests in mango crops in Brazil. The objective of the present work was evaluate the fruit fly population dynamic and diversity in an mixed orchard of Haden, Tommy Atkins and Palmer, in Presidente Prudente, SP, Brazil.

Methods: From August 2005 to December 2007, flies were captured weekly in nine yellow McPhail traps. Inside the trap we disposed 400 mL of 5% hydrolisated protein (Isca Tecnologias Ltda.), renewed weekly.

Results: We collected a total of 65,956 flies: 2,788 of *Anastrepha* spp. (4.23%) and 63,168 of *Ceratitis capitata* Wied. (95.77%). From the *Anastrepha* spp. specimens, 55.45% are males and 44.55% females. For medfly, we captured 43.69% males and 56.31% females. During 2006 we obtained 85.65% of *C. capitata* and 94.08% of *Anastrepha* spp. from the captured flies. The following species were identified: *Anastrepha obliqua* (Macquart) (91.92%), *A. fraterculus* (Wied.) (3.24%), *A. sororcula* Zucchi (1.37%), *A. pseudoparalella* (Loew) (1.11%), *A. striata* Schiner (0.77%), *A. barbiellinii* Lima (0.26%), *A. daciformis* Bezzi (0.09%), *A. haywardi* Blanchard (0.09%), *A. leptozona* Hendel (0.60%), *A. zenildae* Zucchi (0.09%) and *A. montei* Lima (0.85%). Only *A. obliqua* was considered constant species.

Conclusions: During the monitoring *C. capitata* was dominant. Among eleven *Anastrepha* species, only *A. obliqua* was constant and dominant. Mango is a common host for *Ceratitis capitata*, *A. fraterculus* and *A. obliqua* in the State of São Paulo.

Keywords: Anacardiaceae, population dynamic, Tephritidae, faunistic analyses.

PROLONGED PUPAE DIAPAUSE AFFECTS VITAL FITNESS COMPONENTS OF *RHAGOLETIS CERASI* ADULTS.

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Background: Diapause is a physiological process allowing insects to exploit seasonally fluctuating resources, diversify, and colonize harsh environments. Although several physiological and ecological aspects of diapause have been studied in detail, little is known regarding the effects of prolonged diapause on adult life history traits. The European cherry fruit fly, *Rhagoletis cerasi* L. (Diptera: Tephritidae) is a univoltine species undergoing obligatory pupae diapause for, usually, one or more years. Thus, it forms an excellent model organism to test whether prolonged pupae diapause tradeoffs adult fitness components.

Methods: In 2007-2009, we studied the demography of *R. cerasi* adults of three Greek populations, emerging from pupae that terminated diapause after one (1st year) or two (2nd year, prolonged diapause) cycles of high-low-high temperatures. The three populations were originating from: i) Mikra (Thessaloniki), ii) Kamari (Magnisia), and iii) Kernitsa (Achaia). In all populations, pupae, recovered from field infested, ripe sweet cherries, were placed at 25°C for 2.5 months, then at 3°C for 6 months, and finally at 25°C until adult emergence (1st year adults). Overlying pupae were held at 25°C for 2.5 months, and then at 3°C for an additional period of 6 months, and ultimately, returned to 25°C until adult emergence (2nd year adults). A pair of newly emerged adults (one male and one female) were held into individual cages containing oviposition substrates (five ceresin domes), standard adult diet and water. Fifty such pairs (replicates) were established for each population and diapause regime. Daily egg production and female and male ages at death were recorded under constant laboratory conditions (25 ± 1°C, 65 ± 5% R.H., and L14:D10). Additionally, the size of the body of each adult (female and male) was assessed at death by measuring thorax length and width, and head width. Somatometric data showed that both males and females emerging after prolonged pupal diapause were larger than their counterparts emerging the first year. Average longevity, for both males and females, differed significantly among populations the first year, but not among adults emerging after prolonged pupae diapause. Within each population, average longevities of adults emerging the second year were either similar or longer than of those emerging the first year. Average fecundity was higher for females emerging the first year than that of their second year counterparts. Fecundity varied substantially among the different populations the first year (260 – 380 eggs/female), but it was similar after prolonged pupae diapause (≈100 eggs/female). Oviposition and postoviposition periods of females emerging the second year were 15 and 5 days shorter and longer, respectively, than that of females emerging the first year.

Conclusions: Collectively, these data suggest that prolonged pupae diapause: i) is associated with larger adult body size, ii) decreases lifetime female fecundity rates, iii) alters the age specific patterns of oviposition, and iv) does not substantially affect the adult lifespan.

Keywords: European cherry fruit fly, life history traits, demographic parameters, body size, fitness cost, Tephritidae

THE INFLUENCE OF HOST FRUIT AND TEMPERATURE ON THE BODY SIZE OF ADULT *CERATITIS CAPITATA* (WIEDEMANN) IN THE LABORATORY AND IN FIELD POPULATIONS.

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Background: The adult body size of medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), varies in natural conditions. Both temperature during larvae development and host fruit quality have been cited as possible causes for this variation. Body size is an important fitness indicator in insects; larger individuals usually exhibit increased survival, longevity, mating success and fecundity. We studied the influence of host fruit and temperature during larvae development in adult body size in field populations of medfly with the aim to quantify body size variation throughout the year.

Methods: The size of adult *C. capitata* was measured and analyzed using wing surface area as an estimate of adult size. Flies measured had three origins. Firstly, flies periodically collected along the year in field traps from 32 groves in Spain, during the period 2003-2007. Secondly, flies evolved from field collected fruits. Finally, flies reared in laboratory on different foods and in different temperature regimes.

Results: In the lab, wing area of male and female adult medflies varied significantly with developing temperature of larvae, being larger at the lowest temperature (females: $F = 114.06$; $df = 4, 209$; $P < 0.0001$, males: $F = 107.89$; $df = 4, 209$; $P < 0.0001$). Adult size was also significantly different depending on host fruit in which larvae developed (females: $F = 11.23$; $df = 3, 127$; $P < 0.0001$; males: $F = 7.84$; $df = 3, 131$; $P = 0.0001$). Females developed in apricot were the largest followed by those developing in peach, plum and orange. The size of the flies captured at the field varied seasonally showing a gradual pattern of change along the year. The largest individuals were obtained during winter and early spring and the smallest during late summer. Size of adult flies showed less variation according to host fruit in which larvae developed in the field.

Conclusions: Adult size in field populations of *C. capitata* changes periodically influenced mainly by temperature, and secondarily by species of host fruit. These observations could improve our current background on the behavior of *C. capitata* in the field. The importance of final adult size and its implication in the sterile insect technique are discussed.

Keywords: medfly, body size, host, temperature-size rule.

FRUIT FLIES OF ECONOMIC IMPORTANCE WITH SPECIAL REFERENCE TO *BACTROCERA CUCURBITAE*, A GLOBAL REVIEW.

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Background: Tephritids are one of the most destructive and diversified group of insects. There are more than 4000 species of fruit flies in the world in 500 genera which the melon fly *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) is an important pest of Cucurbitaceae (melon, watermelon, pumpkin, squash) in different countries such as most Asian countries, Middle East and Hawaii. This species is responsible for both direct economic losses in vegetable production and for the considerable efforts implemented for the detection and eradication programs in those countries where this pest is found. Also fruit flies of the family Tephritidae are considered the most important quarantine insect pests in terms of international trade. Chemical control, first used as the only management strategy, led to insect resistance, high cost and contamination of fruits and the environment and negative effect on beneficial arthropods. Due to this reason, in recent years several tactics and strategies such as sterile insect techniques, bait spray, Biological controls, legislative control and pheromone traps, alone or in combination have been used. However, in spite of the tremendous investments and efforts made to date, the pest has not been pushed out of all invaded areas and the war is far from being won. In this study, we reviewed several control strategies and focus on success or failure of each method.

Keywords: Melonfly, *Bactrocera cucurbitae*, Sterile Insect Technique, Pheromones

TEPHRITIDAE ASSOCIATED WITH MANGO DAMAGES IN THE WEST OF BURKINA FASO: IDENTIFICATION AND IMPORTANCE OF THE DAMAGES.

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Background: Mango fruits export constitutes an important source of currency for Burkina Faso. However, the damages inflicted by Tephritidae (fruits fly) to mangos threaten the viability of this sector. Poor knowledge of fruits fly species responsible for these damages makes difficult the development of an adapted control strategy. The present study aims to identify the Tephritidae species associated with these damages and to assess their importance on eight cultivars. Two hypotheses were formulated i) The damages caused by Tephritidae on the mango in the West of Burkina are caused by several fruits fly species and, ii) The infestation rate of the mangos by Tephritidae varies significantly depending on the cultivar.

Methodology: Fruits of six grafted cultivars (Amélie, Brooks, Keitt, Kent, Lippens and Springfield) and two non-grafted ones (Mango vert and Sabre) of mango (*Mangifera indica*) served as plant material. During mango seasons 2008 and 2009 (March to August), a sample of 36 fruits per cultivar was taken every 2 weeks from eight orchards. Fruits were incubated using the method described by Vayssières *et al.* (2004), so as to determine infestation rates. Pupae collected from the infested fruits allowed after hatching, identification of fruits fly species (Diptera: Tephritidae) associated with the damages. The analysis of variance at 5 % threshold after data transformation allowed comparison of the infestation rates.

Results: Seven species of Tephritidae of the genus *Bactrocera* (1species) and *Ceratitis* (6 species) were identified. *B. invadens* and *C. cosyra* appeared from most of the infested fruits (61.14 % and 36.1 % of the hatched adults, respectively). The rates of mango infestation varied between 2.4 % for Springfield at the beginning of the season (April, 2008) and 69.2 % for Brooks at the end of the season (August, 2008). The comparison test of the averages has distinguished five classes of cultivars according to infestation rates ($F = 6.181$; $P = 0.0001$). Amélie was the grafted cultivar most weakly infested whereas Keitt was found to be most strongly infested.

Conclusions: This study has demonstrated (1) the existence of seven Tephritidae species infesting the mango among which *B. invadens* and *C. cosyra* are the most associated with the damages and (2) the significant influence of cultivars on the rate of mango infestation by Tephritidae. These results suggest a much better comprehension of the biology and ecology of these two species in the study zone for a more effective control strategy of fruits fly damages.

Keywords: *Bactrocera invadens*, *Ceratitis cosyra*, *Mangifera indica*, quarantine insects, infestation rate.

NATURAL FRUIT FLY INFESTATION IN STONE FRUITS IN THE STATE OF SÃO PAULO, BRAZIL.

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Background: Fruit fly species (Diptera: Tephritidae) were considered key pests in Rosaceae crops in Brazil, causing fruit losses and increasing its costs due to cover insecticide sprays. In the case of peaches, the growers provide additionally fruit bagging after reaching 2.0 cm in diameter. We report in the study the fruit fly species recovered from peaches, nectarines and plums in the southwest region of the São Paulo, Brazil.

Methods: The study was conducted in the Germoplasm Bank installed at the Experimental Station of Agência Paulista de Tecnologia dos Agronegócios, in the municipality of Capão Bonito. During 2004, 2005 and 2006, mature fruits from 34 varieties were picked up at random from the canopy of unsprayed trees. The samples were bring to the laboratory and each fruit were placed on sieved sand in an isolated plastic container covered by a fine mesh cloth. Fruit fly puparia were extracted from the sand with a sieve and kept in a small cage until adult emergence.

Results: Except 'Harry Pieckstone', all remainder varieties were infested by Tephritidae: 'Aurora 1', 'Flor da Prince', 'Douradão', 'Dourado 1', 'Dourado 2', 'Ouromel 2', 'Aurora 2', 'Joia 1', 'Joia 2', 'Joia 4', 'Regis', 'Ouromel 3', 'Centenária', 'Rubrosol', 'Diamante', 'Rosalina', 'Brasão', 'Josefina', 'Reubennel', 'Januaria', 'Grancuore', 'Gema de Ouro', 'Roxa de Itaquera', 'Kelsey 31', 'Kelsey Paulista', 'Tropical', 'Carmesin', 'Fla 680/13', 'Fla 87-7', 'Fla 87-1', 'Okinawa' and Mume (*Prunus mume*). From a total of 1,454 puparia of Tephritidae emerged 1,328 adults. We obtained only 669 *Ceratitis capitata* Wied. (361 females and 308 males) and 659 *Anastrepha fraterculus* Wied. (302 females and 357 males).

Conclusions: *Ceratitis capitata* and *A. fraterculus* are the most poliphagous fruit fly species in the state of São Paulo. Consequentelly, the IPM strategies in stone fruit crops depend on the knowledge of suitable hosts for both fruit fly species in the local agroecosystem.

Keywords: medfly, south-american fruit fly, Rosaceae; peach, plum

WOLBACHIA IN THE ORIENTAL FRUIT FLY *BACTROCERA DORSALIS* (DIPTERA, TEPHRITIDAE) IN PAKISTAN.

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Background: *Wolbachia* are a common and widespread group of endosymbiotic bacteria which is capable of manipulating reproduction of arthropod and nematode hosts. This bacterium causes a variety of reproductive abnormalities including cytoplasmic incompatibility CI. *Wolbachia* has been recently reported as a possible means for insect pest population control. *Bactrocera dorsalis* is an important economic pest of orchards and vegetables and is found in Asia including Pakistan. A *Wolbachia* strain was identified in 19 specimens analyzing over 1500 *Bactrocera dorsalis* individuals from China. Here we report on the identification of a different *Wolbachia* strain from a Pakistani *B. dorsalis* population.

Methods: Collection of *B. dorsalis* adults was done from two regions in Pakistan. DNA was extracted from individual adult flies. Analysis was done by PCR using general *wsp* primers, cloning and sequencing. Individuals without visible amplicon were tested for low titer infections by Southern blot.

Results: In most *B. dorsalis* individuals a PCR amplicon was detected using the general *wsp* primers. Comparison of the *wsp* sequences showed differences between Pakistan and China specimens. Analysing the individuals without visible amplicon by Southern blot revealed a 100% *Wolbachia* infection rate in Pakistan.

Conclusions: All individuals analyzed revealed to be infected by one *Wolbachia* strain contrasting the data of the Chinese population. MLST markers will be applied to this new *Wolbachia* strain to bring more insight into its phylogenetic relationships.

Keywords: *Bactrocera dorsalis*, *Wolbachia*, Pakistan, phylogeny, *wsp*

COLOR AND VOLATILES RESPONSES OF FEMALE *BACTROCERA DORSALIS* (HENDEL) (DIPTERA: TEPHRITIDAE) TO DIFFERENT MANGO RIPENING STAGES.

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Background: Tephritid females locate their host plants using both visual and olfactory cues. The color and volatiles preferences of female fruit flies tend to relate to their host fruits. The responses of female fruit flies to fruit abundance are partially the consequence of the stimulation by host fruit volatiles and physiological stage of female flies. Understanding the visual and olfactory responses of tephritid female flies is important for the development of trap design, trap deployment and orchard design strategies.

Methods: This study isolated host visual and olfactory cues for three mango ripening stages. The artificial mangoes of the three mango ripening stages (unripe, ripe and fully-ripe) were used to study the effect of color to host preference of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). A Y-tube olfactometer was used to determine the preference of *B. dorsalis* female flies on volatiles of each mango ripening stage. A 21-22-day-old, gravid female *B. dorsalis*, which had no prior oviposition experience, were used to study on color and volatile preferences. All experiments were carried out between 07:00-15:00 hours.

Results: Analysis did not detect a significant differential response by female flies to the three different ripening stages of artificial mango (female response to unripe fruit = 23 flies; ripe fruit = 31; fully-ripe = 36; $\chi^2 = 2.867$, d.f. = 2, P = 0.239, n = 90). Many female flies were observed attempting to oviposit in artificial mangoes after arrival at fruit. Female flies showed a significant preference to the volatiles of fully-ripe mango over the volatiles of ripe mango ($\chi^2 = 11.267$, d.f. = 1, P = 0.001, n = 60) and unripe mangoes ($\chi^2 = 48.60$, d.f. = 1, P < 0.0001, n = 60). When female flies were presented with the volatiles of ripe and unripe mangoes, female flies significantly preferred the ripe mango volatiles over the unripe mango volatiles ($\chi^2 = 48.60$, d.f. = 1, P < 0.0001, n = 60).

Conclusions: Host fruit color played only a minor role in host quality assessment by *B. dorsalis* female flies, whereas host fruit volatiles played an important role in the determination of host quality. Many female fruit flies tried to oviposit into artificial mango demonstrates that color and shape of host fruit alone can stimulate oviposition behavior of female fruit flies.

Keywords: artificial mango, *Bactrocera dorsalis*, host fruit, oviposition, Y-tube.

FRUIT FLY SPECIES (DIPTERA: TEPHRITIDAE) DIVERSITY IN THREE REMNANT AREAS OF THE HIGHLY ENDANGERED ATLANTIC RAINFOREST IN THE STATE OF BAHIA, BRAZIL.

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Background: The Atlantic rainforest in Brazil is considered one of the richest biomes on earth and southern Bahia harbors one of the few and largest remnants of the highly endangered forest and an important center of endemism.

Methods: This study was carried out in three remnants of the Atlantic rainforest in the municipalities of Belmonte, Ituberá, and Una, in the state of Bahia, for two years. We documented the diversity and dynamics of the three most frequent fruit fly species. Fruit flies were collected using 10-12 plastic McPhail traps baited with hydrolyzed protein and set up in tree canopies for each fragment.

Results: A total of 7,470 fruit fly adults was captured (4,376 females and 3,094 males). Seventeen *Anastrepha* species were recorded, *Anastrepha antunesi* Lima, *Anastrepha bahiensis* Lima, *Anastrepha dissimilis* Stone, *Anastrepha distincta* Greene, *Anastrepha fraterculus* (Wied.), *Anastrepha furcata* Lima, *Anastrepha grandis* (Macquart), *Anastrepha leptozona* Hendel, *Anastrepha minensis* Lima, *Anastrepha obliqua* (Macquart), *Anastrepha pickeli* Lima, *Anastrepha pseudoparallela* (Loew), *Anastrepha quinae* Lima, *Anastrepha serpentina* (Wied.), *Anastrepha simulans* Zucchi, *Anastrepha sororcula* Zucchi, and *Anastrepha zenildae* Zucchi. *Ceratitis capitata* (Wied.) specimens were trapped only in the fragment in Belmonte. *A. minensis* and *A. simulans* are reported in the state of Bahia for the first time. In the fragment in Belmonte, *A. fraterculus* showed two population peaks in December 2007 and August 2008, whereas *A. leptozona* and *A. distincta* showed a population peak in April de 2008. In the remnant in Ituberá, *A. distincta*, *A. bahiensis*, and *A. fraterculus* showed a population increase from March to June 2008, *A. distincta* showed a population peak in April and *A. bahiensis* and *A. fraterculus* in May. In the fragment in Una, few specimens were trapped during the study period and *A. fraterculus* showed a population peak in February and April 2008, *A. distincta* and *A. antunesi* showed a small population increase in April 2008. In the three fragments, the capture of flies was restricted to a few months during the study period coinciding with the host fruiting period.

Conclusions: This is the first fruit fly survey in the Atlantic rainforest in Bahia. Our results elevated to 33 the number of *Anastrepha* species registered in the state. Most *Anastrepha* species recorded were monophagous or oligophagous.

Financial support: FAPESB, Plantações Michelin da Bahia, CNPq and UESC.

Keywords: *Anastrepha*, population fluctuation, Atlantic rainforest.

FRUIT FLIES (DIPTERA: TEPHRITIDAE) AND PARASITOIDS ASSOCIATED TO *SPONDIAS MOMBIM* L. IN UNA, BAHIA, BRAZIL.

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Background: Yellow mombim (*Spondias mombim* L.) fruit have a very characteristic flavor and are greatly appreciated in Brazil. In southern Bahia, the collection of this fruit has been a source of income for local producers who produce frozen concentrate juice for the domestic market. There are no studies regarding levels of infestation by fruit flies or control methods for this Anacardiaceae species in southern Bahia, which may be attributed to the fact that the fruits are still obtained in an extractive way. This study aimed at identifying the fruit fly species and associated parasitoids that use yellow mombim ('cajá') as a host.

Methods: We carried out a survey from June 2005 to June 2007 collecting fruit samples weekly from tree canopies and from the ground on an experimental 1 ha orchard in Una, southern Bahia, Brazil. The fruit were individually placed in plastic vials covered with cloth to avoid reinfestation until adults and parasitoids emerged. We determined infestation rates, pupal viability, emergence rates, parasitism rates and fruit loss due to infestation.

Results: A total of 1,003 fruits (13.18 Kg) were collected from which 729 puparia were obtained. A total of 331 tephritid adults and 183 parasitoids emerged, representing a pupal viability of 70.50%. The mean infestation rate was 0.73 puparia/fruit and 55.3 puparia/Kg of fruit. The mean emergence rate was 45.4%. Only two *Anastrepha* species were recovered from the infested fruit, *Anastrepha obliqua* and *Anastrepha antunesi*. *A. obliqua* showed a 78% emergence rate and *A. antunesi* showed a 22% emergence rate. The parasitism rate recorded was 35.6%. Three braconid species emerged from *Anastrepha* larvae/puparia, *Doryctobracon areolatus* and *Utetes anastrephae* (Opiinae) and *Asobara anastrephae* (Alysiinae). Parasitoids in the subfamily Opiinae showed a frequency of 84% and the only species in the subfamily Alysiinae showed a frequency of 16%. *D. areolatus* was the most frequent parasitoid associated to *A. obliqua* and *A. antunesi*. Fruit loss ranged from 11% to 93.4%. Infestation rates over 87.54 puparia/Kg of fruit corresponded to more than 50% of fruit loss.

Conclusions: Our results indicate that *A. obliqua* and *A. antunesi* damage yellow mombim fruit during the period of highest fruit production and that any action directed at controlling these fruit flies in the field should be applied prior to or during this period.

Financial support: from CNPq, FAPESB, and UESC.

Keywords: *Anastrepha*, *Spondias mombim*, parasitoids.

THE CURRENT KNOWLEDGE ON *ANASTREPHA* SCHINER (DIP., TEPHRITIDAE) IN THE STATE OF AMAPÁ, BRAZIL.

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Background: Until the 1990s, studies with fruit flies in the state of Amapá located in northern Brazil were virtually nonexistent. However, studies were intensified after the official detection of the carambola fruit fly (*Bactrocera carambolae*) in Oiapoque border with French Guiana in 1996. *B. carambolae* is a quarantine pest for Brazil and is restricted to the state of Amapá, under official control of the Brazilian government. This study aims to list the species of fruit flies of Amapá, highlighting those that have no known host.

Methods: In recent years, periodic and intensive surveys of fruits (cultivated and wild species) have been conducted in almost all counties of Amapá, generating a significant amount of information on the diversity of fruit flies and host plants.

Results: Currently 32 species of the genus *Anastrepha* are recorded for Amapá. Of these, 17 species are known hosts: *A. anomala*, *A. antunesi*, *A. atrigona*, *A. bahiensis*, *A. coronilli*, *A. distincta*, *A. fraterculus*, *A. hastata*, *A. leptozona*, *A. obliqua*, *A. parishi*, *A. pseudanomala*, *A. serpentina*, *A. sororcula*, *A. striata*, *A. turpiniae* and *A. zenildae*. For 15 species there is no host record in the state of Amapá by having been captured only in McPhail traps: *A. amita*, *A. binodosa*, *A. dissimilis*, *A. duckei*, *A. flavipennis*, *A. furcata*, *A. limae*, *A. minensis*, *A. mixta*, *A. pickeli*, *A. pseudoparalella*, *A. shannoni*, *A. sodalis*, *A. submunda* and *Anastrepha* aff. *mucronota*. The diversity of tephritid species probably is related to the diversity of fruit species in the state. On the other hand, little is known about the influence of meteorological factors on tephritid populations in the region.

Conclusion: Despite reasonable knowledge about the Tephritidae diversity in the state of Amapá, the knowledge about host plants and natural enemies is still scarce, especially studies on biology and population ecology.

Keywords: Fruit fly, *Bactrocera*, diversity, survey, Amazon.

AMAZON NETWORK FOR RESEARCH ON FRUIT FLIES.

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Background: Little is known about the diversity of fruit flies species (Diptera: Tephritidae); reported as one of the main pests affecting agriculture worldwide, in addition to with the low number of researchers working on this biological group in the Amazon region, it motivated the structuring of a research project in the Amazon to allow the integration between institutions and generation of information on the families Tephritidae and Lonchaeidae. With funding from the Brazilian Agricultural Research Corporation – Embrapa was create the "Amazon Network for Research on fruit flies" led by Embrapa Amapá (August/2007 to July/2010). Bringing together researchers from research institutions in 10 Brazilian states, the Network aimed to generate and spread information on the diversity, distribution, host plants and natural enemies of fruit flies in the Amazon, with emphasis on the carambola fruit fly (*Bactrocera carambolae*), quarantine pest, restricted to the state of Amapa and under official control of the Ministry of Agriculture, Livestock and Supply. The research priorities on *B. carambolae* were established by the Scientific Committee of the National Eradication Program of carambola fruit fly, aiming at to support the actions in the state of Amapá.

Methods: Samplings of fruits and surveys with McPhail traps in various states of the Brazilian Amazon were performed. In the laboratory, fruits were processed according to the methodology used to obtain fruit flies.

Results: As results of this project were obtained new records of fruit flies (Tephritidae) and "lance flies" (Lonchaeidae) species, host plants and parasitoids in the Brazilian Amazon and Brazil, generating a significant advance in scientific knowledge on these flies. Team members were also trained mainly in the taxonomy of fruit flies. New research projects were approved in the network, emphasizing knowledge of fruit flies species of economic importance.

Conclusion: The greater impact of the scientific network will be obtained at the end of 2010 with the book "Fruit flies in Brazilian Amazon: diversity, hosts and natural enemies", which will present the innovative results added to the knowledge previously available in the literature.

Keywords: Tephritidae, Lonchaeidae, *Bactrocera carambolae*, diversity, host.

CUCURBITACEAE HOSTS FOR NATURAL INFESTATION OF *ANASTREPHA GRANDIS* (MACQUART) IN THE OF SÃO PAULO STATE, BRAZIL.

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Background: *Anastrepha grandis* (Macquart) is the most important pest of cucurbits in Brazil due to the direct damages and restrictions for its exportation. Growers from the western region of the State of São Paulo export squash under the System Risk Mitigation for *A. grandis*. The objective of the present study was investigated the infestation of *A. grandis* in cucurbits in the remainder regions of the State of São Paulo.

Methods: From April 2009 to March 2010, 153 cucurbit fruit samples were collected from 22 municipalities. The samples were brought to the laboratory, weighed and kept into plastic box containing a thin layer of vermiculite and covered with cotton cloth. The fruits were kept for about 30 days. The pupa were transferred to the glass cup (500 cc) with vermiculite under transparent glass jars (6000 cc) and kept at 25°C±2°C and 70±10% relative humidity until adult emergence.

Results: Only six municipalities have showed cucurbit fruits infested by *A. grandis*: Bauru, Campinas, Ituverava, Monte Alegre do Sul, Platina and Socorro. The following species and respective cultivars showed infestation by *A. grandis* (pupae/fruit; pupae/kg of fruit): *Cucumis sativus* 'Caipira' (14.5; 29.2), *Cucurbita maxima* 'Exposição' (55.9; 40.0), *Cucurbita moschata* 'Caravela' (84.7; 21.4), *Cucurbita moschata* 'Menina Brasileira' (38.4; 17.7), *Cucurbita moschata* 'Canhão' (5.0; 0.9), *Cucurbita pepo* 'Caserta' (29.5; 46.6) and hybrid *C. moschata* x *C. maxima* 'Tetsukabuto'. No infestation was registered on *C. moschata* 'Paulistinha'; *C. moschata* 'Goianinha'; *Cucurbita mixta* 'Caipira'; *Lagenaria siceraria* and *Cucumis* sp.

Conclusions: Cucumber (*Cucumis sativus*), squash (*Cucurbita maxima* and *Cucurbita moschata*), pumpkin (*Cucurbita pepo*) and the hybrid squash (*C. moschata* x *C. maxima*) are suitable hosts for *A. grandis* in the State of São Paulo, Brazil.

Keywords: South American Cucurbit Fruit Fly, *Cucumis*, *Cucurbita*, *Lagenaria*, infestation.

FRUIT FLY ON CHILLI IN THAILAND: SPECIES AND ITS BIOLOGY.

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Background: Fruit fly (Diptera: Tephritidae) is the major insect pests of chilli cultivation in Thailand. The Solanum fruit fly, *Bactrocera latifrons* (Hendel) is known as an insect pest of Solanaceae crop. This species is distributed throughout South-East Asia, Taiwan, India and southern China. We studied on species and biology of fruit flies on chilli plantation.

Methods: Study on fruit fly species and its biology on chilli were conducted both in the laboratory and field conditions during 2005-2008. Monitoring and collection fruit fly species on chilli were carried out in Nakornpatom, Kanchanaburi, Bangkok, Ubon-Ratchathani, Khon-Kaen and Chaiyaphum provinces during December 2005 - August 2008. The damaged chilli fruits were collected and kept in the laboratory until fruit flies emerged for identification. The conditions of laboratory were 23.95 ± 0.82 °C and $90.24 \pm 2.635\%$ relative humidity (RH).

Results: Remarkably, we found only the Solanum fruit fly, *B. latifrons* at the number of 4,551 individuals with sex ratio 1:1 from the total number of 19,779 infested chilli. Biological study of *B. latifrons* was conducted in the laboratory during 2005-2008. Adult females laid their eggs singly after her preoviposition period of eight days. Egg stage lasted 44-68 hours with the average of 192.17 ± 75.18 eggs/female. Percentage of egg hatching was 88%. Larval development period was three instars. Total larval development period was ranged 8-10 days and averaged 8.76 ± 0.71 days. Pupal period averaged 11.97 ± 0.77 days with the range of 11-14 days. The longevity of adult female and male were averaged 147.90 ± 29.03 and 131.50 ± 12.79 days, respectively. Life table study of *B. latifrons* in bush red pepper revealed that the appearance mortality of the first instar was remarkably high up to 31.82% but the third instar was low to 10.20%, whereas the survival rate was decreased but stage and age were increased

Conclusion: The results revealed that the Solanum fruit fly, *B. latifrons* is the only one fruit fly species infested on chilli in Thailand. Life cycle of *B. latifrons* was 23.56 ± 0.98 days and the survival rate of egg to adult was 33% under laboratory conditions. Egg parasitoid, *Forpius arisanus* (Sonan) (Hymenoptera: Braconidae) and larval parasitoid, *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) were found to be the natural enemies of *B. latifrons*.

Keyword: biology, Solanum fruit fly [*Bactrocera latifrons* (Hendel)].

POPULATION OF CHILI FRUIT FLY AND ITS DAILY ACTIVITY IN CHILI PEPPER (*CAPSICUM ANNUM*) CROP IN LEMBANG WEST JAVA - INDONESIA.

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Background: Chili fruit fly is one kind of pest which attack chili pepper fruit (*Capsicum annum*) which has economic impact. However, in Indonesia, the species of *Bactrocera dorsalis* (complex) always present on the same localities. Many control measure has been apply to control the population such as application of attractant. More than twenty sibling species are attracted to methyl eugenol. In order to control Chili fruit fly, we need the information on the species and its behavior. The objectives of this research are to identify fruit fly species from chili pepper, daily activity and its damages in the field.

Methods: Chili fruit fly collection was done from chili invested fruit and by methyl eugenol trap at Lembang, West Java - Indonesia. Morphological character was measured to confirm species identification such as: male & female genital organ of fruit fly from infested fruit and from trap caught. Daily activity of fruit fly in the field was observed every hour using methyl eugenol trap. Fruit fly damage on chili pepper was observed in 20 chili plants in the field.

Results & Conclusions: From invested fruit, the length of aculeus was about 1.9 - 2.19 (2.02 ± 0.09) micron, aedeagus length was about 3219 – 3853 (3534.7 ± 192.9) micron. However the length of male fruit fly aedeagus caught from the trap was about 3195 – 3975 (3658.3 ± 156) micron. It was concluded that fruit fly species from chili pepper was *Bactrocera papayae*. Male's fruit fly respond to methyl eugenol trap in the field showed significant activity at 08.00 to 15.00 and the highest activity was at 11.00 to 14.00. From 20 chili plants, almost 100% of chili plant was damage by chili fruit fly and the intensity of damages for each plant varied between 4-94%.

Keywords: *B papayae*, *Capsicum annum*, fruits damage, morphological character.

COMPARISON STUDY OF VOLATILES PRODUCED BY CALLING MALE OF *CERATITIS CAPITATA* FROM DIFFERENT POPULATIONS.

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Background: *Ceratitis capitata* is one of the most widespread member from the fruit fly family (Tephritidae). There have been numerous SIT (Sterile Insect Techniques) programs running all around the world to control natural populations of this pest. In these programs, males originating from mass produced laboratory colony are sterilized by irradiation and released into the field. It is supposed that sterile males which mate with wild females generate no progeny. The efficiency of these programs is based on the assumption that laboratory males reared on artificial diet are able to compete with wild males. In fruit flies, mating behavior is quite complex and involves chemical, visual and acoustic communication. Chemical communication is based on sex pheromone released by males to attract females.

Methods: In order to provide adequate studies concerning compatibility or incompatibility of laboratory and wild populations of *C. capitata*, we analyzed and compared the chemical composition of volatiles released by calling males of one laboratory and two wild populations of *C. capitata* using GC×GC/TOFMS technique and Principal Component Analyses (PCA).

Results: Our results indicate that there is a strong correlation between laboratory and wild populations of *C. capitata* studied, suggesting that males from these populations use the same chemical language to attract females.

Conclusions: These results are of significance for the successful implementation of the SIT program.

Keywords: Medfly, pheromone, populations, SIT, GC×GC/TOFMS, PCA

EMBRYONIC DEVELOPMENT OF *ANASTREPHA LUDENS* (LOEW).

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Background: The egg stage and the embryonic development of *Anastrepha ludens* is one of the less studied despite the great importance for mass rearing facilities where it is necessary obtain high percentages of egg hatch. Peculiar mechanisms of elimination of yolk during gastrulation and final head lobules development has been described for other *Anastrepha* species. However *A. ludens* has not been studied previously. We describe the different egg stages and time during embryonic development and the emergency process. This knowledge contributes to the developmental embryonic of this species and is useful in early evaluation of egg hatch, which is a valuable date to calculate egg density and at the moment of seeding.

Methods: Samples of eggs of a colony maintained in the laboratory during generations, named “Chiapas”, were collected in oviposition panels with water during 30 minutes; they were incubated in petri dishes with a humid filter paper and maintained in a bioclimatic chamber at 26° C. Observations with a transmitted light stereomicroscopy were made each two hours. It was not necessary to remove the chorion, because the egg covers are transparent. It was identified the anterior and posterior poles and registered the moment when appear o disappear the yolk masses. It was observed the movements of larvae near the emergence and identified the place where it hatches.

Results: The morphological changes of the egg were monitored during the embryonic development. Based in yolk masses extruded during the embryonic development of *A. ludens* four types of eggs were identified: Type 1 without mass yolk, Type 2 only anterior mass yolk, Type 3 only posterior mass yolk and, Type 4 anterior and posterior mass yolk. The class 3 and 4 were the most abundant, and corroborated that type 1 and 2 presented embryonic development. It is possible to predict at the third day after oviposition the egg hatch, with reliability of 99%, because the unfertilized eggs are clearly identified. Near the emergence the larvae twist 180° and the majority hatch near the posterior pole.

Conclusions: The egg stages during the embryonic development permit to estimate the maximum egg hatch one day before seeding.

Keywords: mexfly, extrusion of yolk mass, *Anastrepha ludens*, embryogenesis, egg hatch, fertility egg

Session 2
Morphology & Taxonomy

DNA BARCODING AS IDENTIFICATION TOOL IN FRUIT FLY INTERCEPTION AND SURVEYING ACTIVITIES

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Background: DNA barcoding uses the sequences of a particular part of the mitochondrial gene COI as a unique identifier for all zoological species. DNA barcodes can provide a valuable alternative to morphological identification in cases where morphological characters do not provide diagnostic features. Complete taxon coverage is currently not feasible because of the unavailability of specimens for the rarer species. However, for quarantine interception, and field survey and monitoring activities with para-pheromone traps, identification of fruit flies through DNA barcodes could be considered. This activity fits within the TBI (Tephritid Barcoding Initiative) and the flagship project on Diptera of JEMU

Methods: Our study focuses on the African fauna of the main fruit fly genera of economic importance (EI): *Ceratitis*, *Dacus*, *Bactrocera* and to a lesser extent *Capparimyia* and *Trirhithrum*. A DNA barcode library will be established for all EI taxa within these genera, as well as for all taxa regularly encountered in para-pheromone trapping activities in different parts of the continent.

Separate sets of intercepted fruit fly samples of European NPPO's and material recently collected during surveying and monitoring activities in different parts of Africa will be requested. This material will be identified independently based upon morphological characters by a taxonomic specialist, and upon DNA barcodes. The efficiency and reliability of both methodologies will be compared.

Results: Currently, a DNA library has been compiled for more than 120 African taxa. This includes all major EI species belonging to the target taxa, several congeneric species, and outliers of related genera. For widespread species, representatives of different geographical regions were included in order to cover for intraspecific variability. The library also includes more than 75% of all taxa regularly trapped in para pheromone traps in the last 20 years. As test material, samples were obtained from French and UK NPPO's, resulting from fruit fly interception in fruit consignments from the last five years. In addition, recent material from survey programs in western and southern Africa was also obtained. Testing is ongoing but preliminary results show that DNA barcodes can provide an alternative as identification tool, especially with regard to juvenile stages like larvae found in intercepted fruit consignments. Difficulties in species differentiation are mainly encountered in recognized species complexes such as the *Ceratitis* FAR complex (*C. fasciventris*, *C. anonae*, *C. rosa*).

Keywords: DNA barcoding, taxonomy, Africa

COULD BE *ANASTREPHA OBLIQUA* (MACQUART) A COMPLEX OF CRYPTIC SPECIES?

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Background: *Anastrepha obliqua* (Diptera, Tephritidae) is an important pest in Neotropical countries, specially damaging mangoes. This species belong to the *fraterculus* group, within one a complex of cryptic species had been described, especially in the *A. fraterculus* (Wiedemann) *s.l.* Resolution of cryptic species status is important for management tactics like SIT or other advising the pest biology. An artificial rearing of *A. obliqua* has been performed for last 10 years in the “Universidad del Tolima” and two populations were collected from fruits of hobo (*Spondias mombin*) and Mango (*Manguifera indica*) in May 2009. Specimens from mango were reared easily following our protocol; however specimens from hobo can not be rearing under laboratory conditions, suggesting a difference in the biology.

Methods: Mature fruits from hobo (H), mangoes (M) and plum (P) (*Spondias purpurea*) were collected in Prado, Espinal and Guamo (respectively), three neighboring municipalities in the Department of Tolima, Colombia. Previous fruit flies collect from these hosts had been always identified as *A. obliqua*. Adults flies were obtained and third instar larvae from them were taken from artificial diet when specimens were collected from mango or plum, and from papaya for specimens collected from hobo. Measurements of adults (thorax, wings and aculeus) and larvae (long, depth of mandible and long of anterior and posterior spiracles) were done. Number of tubules in the anterior spiracles and hairs in the posterior spiracles were observed too.

Results: Aculeus is longer and thinner in H population (1.54 ± 0.04 mm long, 0.088 ± 0.007 mm wide VS 1.53 ± 0.08 , 0.097 ± 0.006 (M) and 1.50 ± 0.01 , 0.096 ± 0.006 (P)); however the aculeus tip was shorter in H (0.182 ± 0.01 mm VS 0.197 ± 0.006 (M) and 0.189 ± 0.01 (P)). The serrate part was shorter but no wider in the H populations (0.130 ± 0.096 mm long, 0.076 ± 0.007 mm wide VS 0.139 ± 0.00 , 0.076 ± 0.00 (M) and 0.144 ± 0.01 , 0.078 ± 0.005 (P)). The mandible in the larvae was less depth in H population (0.035 ± 0.01 mm) than in other populations (0.080 ± 0.009 (M), 0.076 ± 0.00 (P)) and conspicuous longer (0.272 ± 0.007 mm VS 0.265 ± 0.01 (M), 0.261 ± 0.007 (P)); then, the mandibles ratio is so different (0.128 ± 0.04 (H), 0.304 ± 0.04 (M) and 0.290 ± 0.008 (P)). The anterior spiracles have 11-12 tubules in the H populations and 14-16 in the M and P populations. The posterior spiracles have fewer hairs in the H specimens than in the other two populations (approximately a half).

Conclusions: Morphological differences and the biological rearing behavior of specimens collected from hobo suggest two different species (cryptic species) in the *A. obliqua s.l.* Other studies like karyotypes, molecular or crossing will help to clear these findings and experiments had been performed and started in our University.

Keywords: Morphology, adults, larvae, West Indian Fruit Fly, taxonomy.

SPECIES *ANASTREPHA* SCHINER (DIPTERA: TEPHRITIDAE) “FRUIT FLIES” AND GEOGRAPHICAL DISTRIBUTION IN PERU.

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Background: The growing economic importance of the losses caused by fruit flies has been ongoing concern and justified the Ministry of Agriculture through the National Service of Agrarian Health (SENASA). In Peru, where he performed work for the control, suppression or eradication of fruit flies, you need a reliable practice of identifying, measuring and evaluation of the populations of *Anastrepha*, to demonstrate the presence or absence pest and thus obtain the results for the implementation of Integrated Control work their decline. The main objective of this study was: to know the species of fruit flies present, captured in Mc Phail traps and their geographical distribution in Peru, carrying out this analysis based on morphological characteristics of male and female genitalia.

Methods: The specimens were captured in Mc Phail Traps baited weekly during 2003 January-2009 December, installed in 19 Departments, the flies were preserved in alcohol 70% in sealed bottles labeled and sent monthly to the entomology laboratory for taxonomic identification to species level by analysis of male and female genitalia, which in some specimens were performed for assemblies. For the determination of species it was used the Identification Manual for fruit flies prepared by Korytkowski in 2007.

Results: The results obtained were distributed in 29 species, in alphabetical order these are as follows: *Anastrepha alveata*, *A. atrox*, *A. barnesi*, *A. chichlayae*, *A. curitis*, *A. dissimilis*, *A. distincta*, *A. fraterculus*, *A. flavipennis*, *A. grandis*, *A. hamata*, *A. kuhlmanni*, *A. lambda*, *A. lanceolate*, *A. leptozona*, *A. macrura*, *A. manihoti*, *A. montei*, *A. nigripalpis*, *A. obliqua*, *A. ornata*, *A. pickeli*, *A. pseudoparallela*, *A. schultzi*, *A. serpentina*, *A. shannoni*, *A. striata*, *A. tecta* and *A. willei*, being the most widely distributed species and predominance *A. fraterculus*, considered the most important species, having been found in abundant populations in almost all ecological zones of the region. Of all specimens tested, 51.2% were females and 48, 8% were males, it had a sex ratio of 1:1 approximately.

Conclusions: *Anastrepha* species captured in Mc Phail traps in Peru is 29, but the most common are: *Anastrepha fraterculus*, *A. distincta*, *A. serpentina*, *A. striata* and *A. obliqua*. The most widely distributed species are *Anastrepha fraterculus* and *Anastrepha distincta* and lower distribution is *A. willei* and *A. tecta*. Departments that had great diversity were Cajamarca, Junín, Tumbes, Lambayeque and Huánuco.

Keywords: *Anastrepha*, McPhail Traps, species, fruit flies.

EGG MORPHOLOGY OF *ANASTREPHA* SPP (DIPTERA: TEPHRITIDAE) IN THE *FRATERCULUS* GROUP.

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Background: The *fraterculus* group is the largest and most widespread species group comprising 29 species, 17 of which occur in Brazil. The need to explore additional morphological characters, such as those present in eggs and larvae, besides molecular ones, to better understand the phylogenetic relationships among *Anastrepha* species has been pointed out. Nevertheless, there is a paucity of information on egg morphology and relatively few studies have described the eggs of only 25 species of *Anastrepha*. This paper describes the egg morphology of *Anastrepha antunesi* Lima, 1938, *Anastrepha bahiensis* Lima, 1937, *Anastrepha coronilli* Carrejo & González, 1993, *Anastrepha distincta* Greene, 1934, *Anastrepha turpiniae* Stone, 1942, and *Anastrepha zenildae* Zucchi, 1979 using SEM.

Methods: The length and the width measures of the entire egg were taken with an ocular micrometer and a Wild M3C stereoscopic microscope at Instituto Nacional de Pesquisas da Amazônia – INPA, Manaus, Brazil. In preparation for SEM, eggs were transferred to silicone capsules and dehydrated in an ethanol series, then critical point dried in CO₂ for 3 hours, and sputter-coated with a gold layer. The eggs were examined in a LEO 1450VP scanning electron microscope at Museu Paraense Emílio Goeldi, Belém, Brazil and under an optical microscope (Leica M165C) at INPA. Twenty to thirty five eggs per female of at least three females of each species studied were prepared for light microscopy. SEM was used to examine the chorion in at least 10 eggs.

Results: This SEM study on eggs of six species of *Anastrepha* shows basic similarities to other species whose eggs have already been described as well as some differences previously unreported. *Anastrepha coronilli* and *A. distincta* belong into the category of eggs with a smooth or faintly reticulated chorion – the reticulation in eggs of *A. antunesi*, *A. bahiensis* and *A. distincta* is very faint whereas it is absent in eggs of *A. coronilli*. *Anastrepha turpiniae* and *A. zenildae* are in the category of those species with a sculptured chorion and both species show a very pronounced reticulation.

Conclusions: The results presented here broaden our knowledge of eggshell morphology in *Anastrepha* species in the *fraterculus* group, however more detailed work is needed on additional species to answer important questions regarding phylogenetic relationships within the species groups and the genus as a whole. We expect our results to increase our knowledge of the morphology of *Anastrepha* eggs in the *fraterculus* group and to be useful for taxonomic and systematic purposes.

Keywords: Morphology, fruit flies, Amazon, Brazil

Session 3
Genetics & Evolution

CROSS-AMPLIFIED MICROSATELLITE MARKERS IN THE EUROPEAN CHERRY FLY, *RHAGOLETIS CERASI* (DIPTERA: TEPHRITIDAE), REVEAL AN EXTENDED STRUCTURING OF THE SPECIES NATURAL POPULATIONS IN GREECE.

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Background: The European cherry fruit fly, *Rhagoletis cerasi* (Diptera: Tephritidae), is a major pest of cherries in Europe and parts of Asia. Despite its big economic significance, there is a lack of studies on the genetic structure of its natural populations. Knowledge about an insect pest on molecular, genetic and population levels facilitates the development of environmentally friendly control methods. In this study we present the development of 13 microsatellite markers for the European cherry fly through cross-species amplification and their evaluation through the genotyping of 220 wild flies collected in Greece. Microsatellites constitute very useful tools for population analyses, since they are highly polymorphic, dispersed in the genome, co-dominant inherited Mendelian markers.

Methods: Thirty-nine primer pairs designed for the amplification of microsatellite markers in other Tephritidae species were evaluated for their ability to amplify specific amplicons with *R. cerasi* DNA as template. Those that did so were used for the genotyping of a small sample consisting of 20 individuals. The primer pairs that proved polymorphic, easily scorable and gave reproducible results were used for a preliminary analysis of *R. cerasi* natural populations in Greece.

Results: Thirteen primer pairs conformed to the aforementioned criteria and were used for the genotyping of 220 individual flies, collected from 8 different sampling locations. Two to nine alleles per locus were observed and the vast majority of the markers conformed to Hardy-Weinberg Equilibrium (HWE) in each sample. Genetic distances were very high, pointing towards an extensive structuring of *R. cerasi* natural populations.

Conclusions: Our results indicate that (a) cross-species amplification is a versatile and rapid tool for developing microsatellite markers in *Rhagoletis* spp., (b) the microsatellite markers presented here constitute an important tool for population studies on this pest, and (c) there is clear structuring of natural European cherry fruit fly populations.

Keywords: *Rhagoletis cerasi*, Tephritidae, microsatellites, population genetics, cross-species amplification

THE ROLE OF ESTERASES IN MALATHION AND LAMBDA-CYHALOTHRIN RESISTANCE IN THE MEDFLY *CERATITIS CAPITATA* (WIEDEMANN).

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Background: The insecticide malathion has been widely used in Spain for control of the Medfly *Ceratitis capitata* (Diptera: Tephritidae), and as a result, resistance has developed in field populations. The main resistance mechanism described so far is a point mutation in the acetylcholinesterase gene (*ace2*), the target molecule of malathion, although metabolic resistance mechanisms mediated by esterases may also be involved, as revealed by bioassays with the synergist DEF. In 2009, malathion was withdrawn from the EU market, being mostly replaced by the insecticides spinosad and lambda-cyhalothrin for Medfly control in citrus orchards. We are maintaining in our laboratory a 200-fold malathion-resistant strain, as well as different isolines carrying homozygous mutations in the aliesterase gene *ae7*- which has been previously associated with malathion resistance in *Musca domestica* and *Lucilia cuprina* - although none corresponds to the resistance mutations reported in those species. The malathion-resistant strain is also 5 times less susceptible to lambda-cyhalothrin than the susceptible strain, and has been further selected with lambda-cyhalothrin to give a 40-fold resistant population. Susceptibility in this strain is partially regained with the synergist DEF, which suggests that esterases could also be playing a role in resistance to lambda-cyhalothrin. We have analysed therefore the possible involvement of both carboxylesterases and aliesterases in malathion and lambda-cyhalothrin resistance in *C. capitata*.

Methods: Carboxylesterase specific activity towards the substrate α -naphthylacetate (α NA) has been measured spectrophotometrically in abdomen homogenates of single insects. Aliesterase specific activity towards the model substrate methylthiobutyrate (MTB) has been measured in the same way. Two malathion-resistant strains carrying the point mutation in *ace2*, a lambda-cyhalothrin selected strain and a susceptible laboratory strain, as well as isolines carrying different *ae7* mutations have been analysed.

Results: Enzyme assays revealed that esterase activities were altered in some of the resistant strains and isolines when compared to the susceptible strain. Those populations that showed differences in carboxylesterase or aliesterase activity were further studied by native acrylamide gel electrophoresis (PAGE) using the same substrates, and their isoenzyme profiles compared.

Conclusions: The role of esterases in malathion and lambda-cyhalothrin detoxification is discussed in terms of Medfly control strategies in field conditions.

Keywords: Medfly, insecticide resistance, esterase, aliesterase, malathion, lambda-cyhalothrin

GEOGRAPHICAL DISPERSION OF GLY328ALA ACETYLCHOLINESTERASE MUTATION ASSOCIATED TO MALATHION RESISTANCE IN *C. CAPITATA* (WIEDEMANN, 1824).

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Background: A point mutation in acetylcholinesterase gene (*ace2*) resulting in a Gly328Ala substitution has been associated to malathion resistance in the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann, 1824). In addition, differences in susceptibility among field populations were related to the frequency of the treatments. We have developed a simple PCR-RFLP method useful to detect the resistant allele in field populations, and performed an analysis of the geographical dispersion of this allele among *C. capitata* populations collected in Spain and other countries worldwide.

Methods: Primers allowing a PCR-RFLP method directed to determine the presence of *ace2* gene mutation in genomic DNA have been designed. 20 individuals from 38 *C. capitata* populations (27 from Spanish and 11 from other countries) were analyzed.

Results: Analysis by PCR-RFLP revealed that the resistant allele is widespread all over Spain, being present in 25 from 27 Spanish populations analysed, including populations from different regions such as Comunitat Valenciana, Catalunya, Andalucía, La Rioja, Aragón, Illes Balears and Islas Canarias. The resistant allele was not detected in other populations collected in Guatemala, Brazil, South Africa, Israel, Australia, Morocco, Tunis, Greece, Turkey, Portugal (Madeira Island) and France (La Réunion). The frequency of individuals carrying a resistant allele (in homocigosis or heterocigosis) was related to malathion susceptibility as determined by bioassays.

Conclusions: 1. PCR-RFLP is an effective method to detect the presence of the malathion resistant allele Gly328Ala in field populations of *C. capitata*. 2. Gly328Ala AChE mutation is widespread all over Spain, but is not present in populations from other countries. 3. The frequency of individuals carrying a resistant allele (in homocigosis or heterocigosis) is related to malathion susceptibility determined by bioassays.

Keywords: Medfly, organophosphorus resistance.

POPULATION GENETICS OF TWO FRUIT FLIES DAMAGING CUCURBITS IN LA REUNIÓN: *BACTROCERA CUCURBITAE* AND *DACUS CILIATUS*.

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Background: *Bactrocera cucurbitae* and *Dacus ciliatus* are two fruit fly species damaging various cucurbit crops. Of Asiatic origin, *B. cucurbitae* has become an invasive pest in many countries especially on the African continent. Conversely, *D. ciliatus*, which originates in Africa has increased its distribution during the last years and is now becoming a pest in different Asiatic countries. Both species rank among invasive pests and has been introduced in La Réunion, a French island in the South-Western Indian Ocean over the last 50 years. They now damage the whole range of cucurbits cultivated in the island and cause important yield losses. La Reunion is a subtropical island, with high altitudinal gradient (up to 3000 m) and two seasons (hot and rainy summer, milder and drier winter). Nevertheless, those two fruit fly species are found in most of the areas, regardless of the season and the altitude. However, they show some altitudinal preferences: *B. cucurbitae* is more abundant in the lowlands and *D. ciliatus* at medium altitudes. The aim of this work was to 1) characterize the population structures of *B. cucurbitae* and *Dacus ciliatus*, 2) test host races 3) and seasonal or altitude effects.

Methods: 2500 Individuals of *B. cucurbitae* were collected from 32 sites at high, medium and low altitudes during winter and summer on different host plants all over Réunion island. In order to study the population structure of both species, we developed sets of microsatellite markers for both species, and genotyped at those populations. Genetic differentiations among geographical regions were quantified through hierarchical analysis of molecular variance (ARLEQUIN) and levels of population admixture were quantified through Bayesian clustering procedures (STRUCTURE, TESS).

Results: We obtained 13 polymorphic and specific markers for each species. Those markers were used for genotyping both species. No clear structuration was found for both species according to altitude, season or host plants. Between all sites we had high levels of admixture and reveal patterns of inter-regional, and inter seasonal gene flow.

Conclusions: The low differentiation found between sites among all populations tested is coherent with the recent invasion by one population for both pests into La Réunion. The productions of those 2 sets of molecular markers are a good tool to evaluate small and large scale diversity of both species, and trace back the invasion routes.

Keywords: population genetic analysis, microsatellites, *Dacus ciliatus*, *Bactrocera cucurbitae*.

EXTENSION OF THE PATHWAY ANALYSIS FOR *CERATITIS CAPITATA* (WIEDEMANN) IN THE MEDITERRANEAN AREA.

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Background: The Mediterranean fruit fly (medfly), *Ceratitidis capitata*, is one of the most destructive agricultural pests worldwide. Once introduced, programs to manage or eradicate medfly populations can have tremendous economic costs. To avoid the establishment of this species, non-infested countries (or regions) can apply strict quarantine standards on the import of fresh fruits produced in infested areas. Recently, a worldwide pathway analysis of medfly based on mitochondrial DNA sequences (i.e., a portion of the *cytochrome oxidase I* [COI] gene and a locus including the *NADH-dehydrogenase* subunits 5 and 4 [N5N4] genes) has been published by Barr (2009). This diagnostic tool applies an exclusionary principle to gain knowledge of probable geographic sources of an introduction, considering six predefined geographical regions: Mediterranean area, Central America, South America, Hawaii, Australia and Sub-Saharan Africa.

Methods: In Morocco, the medfly is the most serious pest infesting citrus orchards. In our study, we sequenced both COI and N5N4 markers from Moroccan populations and used the data to assign the Moroccan population to one of the six predefined regions using the exclusionary principle. Moreover, we compare diversity within the country, and update the reference database of medfly diversity. In a second approach to pathway analysis, we applied a SAMOVA (Spatial Analysis of MOlecular VAriance, a without a priori method) on the whole samples (from Barr 2009 and Morocco) to refine the geographical grouping described by the pathway analysis.

Results: For both markers, the most common haplotype among the medfly populations from Morocco supports that they are genetically similar to the Mediterranean region. We found four new haplotypes for COI and eight new haplotypes for N5N4. One COI haplotype is common in Kenya, a probable source for the Moroccan populations. For the more variable marker N5N4, the SAMOVA assumes a subdivision of the Mediterranean unit in two groups, a Western one (Morocco, Spain and Portugal) and an Eastern one (Tunisia, Egypt, Italy, Israel, Greece and Malta).

Conclusions: We conclude that the generation of additional DNA sequences of N5N4 and COI genes for individuals from many regions increases the discrimination power of the pathway analysis tool. The construction of a large reference database from worldwide medfly populations will contribute to identify potential sources of introduction. Such information can help to improve quarantine procedures.

Keywords: Medfly, pathway analysis, mitochondrial DNA, SAMOVA, Mediterranean area

IMPROVEMENT OF GENETIC SEXING STRAINS THROUGH THE INDUCTION OF CHROMOSOMAL INVERSIONS IN *ANASTREPHA LUDENS* LOEW (DIPTERA: TEPHRITIDAE).

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Background: Genetic Sexing Strains (GSS) has been development to be included in the Sterile Insect Technique (SIT) programs. These GSS have been constructed on the basis of induced translocations Y-autosome which allows discriminate and eliminate the females in early development stages so that only males could be released in the field. Because meiotic recombination presented in males, the GSS have a certain percentage of insect aberrant product of recombination between the marker selected for the GSS construction (black pupae *bp*) and the break point of translocation, it is needed to develop colonies in which the phenomenon of recombination is reduced or nullified in its entirety through the induction of chromosomal inversions. For this, it is necessary to have another phenotypic marker linked to *bp* mutation; this new mutant found in 2009 was named “purple iridescence” (*im*), for what is this current work.

Methods: To induced and isolate chromosomal inversions irradiated *bp* males were crossed to *im* females. The resultant F₁ females were then crossed in single pairs to *bp/bp; im/im* double mutant males and the F₂ offspring were screened for families showing absence or a low percentage of recombinant phenotypes. When such families were observed, to produce an homozygous inverted strain, the *bp* males were crossed to *im* females, then the F₃ wild type males and females were inbreeding and finally the homozygous *bp* insects F₄ offspring are in homozygous condition.

Results: The mutation *im* is a monogenetic, autosomal recessive gene characterized by the presence of a bright purple halo in the eyes of the insects easily to identify; these insects also present pupae more elongated and thinner than the wild type. There have been eight trials from witch have been obtained five families carrying chromosomal inversions: Family 4, 154, 63, 79 and 120. Families 63 and 79 showing 3.70% and 3.95% of recombinant phenotypes respectively. The families 4 and 120 presented very weak insects when the chromosomal inversions were in homozygous condition in the F₄ and they could not survive by themselves. Finally, the family 154 was successfully established as a homozygous colony with 500 insects and has not been recovered recombinant insects from this colony.

Conclusions: It was established a recombinant suppressor strain for *A. ludens* using the new phenotypic marker purple iridescence. This chromosomal inversion will help to reduce the recombination frequency in genetic sexing strain Tapachula-7.

Keywords: genetic sexing strains, inversion *A. ludens*

DEVELOPMENT OF A GENETIC SEXING STRAIN FOR *ANASTREPHA LUDENS* USING A BLACK PUPAE (bp^+) TRANSLOCATION IN EGFP/Y-INSERTION MALES.

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Background: In order to improve the sterile insect technique (SIT) several germ-line transformation systems have been developed in pest insects. The Mexican fruit fly, *Anastrepha ludens*, in particular was transformed using *piggyBac* vector constructs, *pXL-PUBEGFP/Asb2t-DsRed.T3*. During this transformation one transgenic line with Y-linked insertion was obtained, expressing EGFP only in males, although the red fluorescence from the testes was not observed. In addition, a black pupae mutation (bp) was previously isolated from the mass rearing MOSCAFRUT facility, and this marker has the potential to create a genetic sexing strain (GSS- bp) by translocating the wild type allele of the mutation (bp^+) to the Y-chromosome. In a bp^-/bp^- mutant strain having the Y- bp^+ translocation, male brown pupae males can be separated from the mutant black pupae females for genetic sexing.

Methods: To improve the male-specific Y-linked marking (and sex-specific selection) in the GSS- bp strain, we translocated autosomal bp^+ allele onto the Y-EGFP chromosome to create Y-EGFP/ bp^+ . Pupae from the Y-EGFP/ bp^+ strain were irradiated with 30 Gy using a Co⁶⁰ source, with subsequent appropriate crosses to select for Y-EGFP/ bp^+ translocations for GSS.

Results and Conclusions: Seven hundred (700) male progeny were screened, from which three potential lines were selected where all females had the mutant *black pupae* phenotype, while all males had the brown pupae (bp^+) wild type phenotype, in addition to green fluorescence observed under epifluorescent optics. These lines were maintained for four generations with no detection of recombinant individuals. One line had fertility higher than 50% and good fitness, which indicates good potential for development into a new GSS line allowing male-specific selection and marking necessary for SIT. This transgenic line is also being tested for remobilization of the vector to a new Y-linked autosomal site that may allow enhanced expression of the EGFP and sperm-specific DsRed markers. Y-linked fluorescence will provide the possibility for male-specific selection, especially if embryonic expression is detectable, for fluorescence-based embryonic sexing.

Keywords: Mexfly, transgenic, genetic sexing strain, *black pupae* mutation, EGFP

MITOCHONDRIAL DIVERSITY OF FIVE TUNISIAN *CERATITIS CAPITATA* POPULATIONS (DIPTERA: TEPHRITIDAE) EVALUATED BY SINGLE STRAND CONFORMATION POLYMORPHISM.

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Background: The Mediterranean fruit fly (*Ceratitis capitata*) (Diptera:Tephritidae) (Wiedemann) is an established pest in regions of north Africa. Little is known on the population structure and dynamics of north African populations. Nonetheless, effective control of this pest depends on knowledge of patterns of colonization of this economically important pest, such as the determination of invasion sources and the elucidation of demographic parameters. The aim of this present work was to analyze the genetic structure of medfly populations in this region, in an effort to understand the colonization process in the area.

Methods: Mitochondrial DNA variation was studied in five natural populations from the northern, north-western, central and southern parts of Tunisia by amplifying three different mitochondrial loci (ND4, ND5 and COII) and subjecting them to Single Strand Conformation Polymorphism analysis (SSCP). The single stranded amplified DNA was electrophoresed on a non-denaturing polyacrylamide gel.

Results: At least three distinct haplotypes were detected. These haplotypes are in the process of additional analysis: amplified bands are being isolated and sequenced in order to determine the exact nature of the detected polymorphisms. Further analysis will also resolve population structuring of Tunisian medfly populations.

Conclusion: SSCP is a convenient, rapid and relatively inexpensive approach of surveying mitochondrial genetic variation. The three loci chosen for the analysis of medfly populations in Tunisia (ND4, ND5 and COII) present substantial sequence variation that can yield in meaningful results.

Keywords : medfly, mitochondrial DNA, SSCP, polymorphism.

TRANSGENIC CONDITIONAL LETHALITY STRAINS FOR THE BIOLOGICAL CONTROL OF *ANASTREPHA* SPECIES.

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Background: A variation of the sterile insect technique (SIT) for the biological control of pest species is the release of transgenic insects carrying dominant conditional lethal genes. Two approaches towards lethality include use of dominant temperature-sensitive (DTS) mutations, and tetracycline (tet)-suppressible lethality systems, in particular an embryo-specific system proven effective in medfly. For DTS lethality in *Anastrepha* spp. we tested a heat-sensitive mutation in the 20S proteasome subunit gene, *Prosβ2*¹, first described in *D. melanogaster*. A missense mutation results in an antimorph at 29°C that disrupts proteasome function, resulting in late larval or pupal death. For tet-suppressible embryonic lethality we have initiated tests of genetic elements isolated from *Anastrepha* species for promoter-driver and lethal effector gene cassettes.

Methods: For DTS lethality, proteasome subunit genes were isolated from *A. suspensa* embryonic cDNA libraries by degenerate PCR, with 5' and 3' genomic sequences isolated by inverse PCR. DTS mutant alleles, created by *in vitro* mutagenesis, were transformed into a wild strain using *piggyBac* transposon vectors. Homozygous lines for the DTS transgene were reared at 25°C and 29°C to evaluate viability from larval to adult life stages. For tet-suppressible lethality embryonic gene promoters and cell death gene cognates were identified from EST sequences by similarity searches and isolated by PCR.

Results: The caribfly *AsProsβ2* cognate was isolated and mutated to create a Gly170Arg substitution (*AsProsβ2*¹). Five transgenic lines homozygous for the mutant transgene pupated at frequencies similar to wild type at 25°C and 29°C, but exhibited pupal lethality rates of 96 to 100% at 29°C. Embryonic gene cognates whose promoters may be used to drive lethality (linked to the tTA transcriptional activator), or from which dsRNA may act as lethal effectors (linked to the tet-response element; TRE) were isolated, including *serendipity* and *slam*. Their promoters were isolated, linked to tTA and are being tested with the TRE-*hid*^{ala5} lethal effector cassette (used in medfly) in *A. suspensa* transformant lines. Potential cell death gene cognates for *hid*, *reaper* and *sickle* were isolated from *A. suspensa* and *A. ludens* and are being tested functionally by cell death assays in *Drosophila* and *Anastrepha* embryonic cell lines.

Conclusions: A DTS conditional lethal strain has been created for *A. suspensa*, yielding complete pupal mortality when reared at 29°C. While highly effective in caribfly, and likely useful in mexfly, post-larval lethality is less than optimal for immediate population control. A tet-suppressible embryonic lethality system, that uses lethal effector genes from *Anastrepha*, will potentially improve upon a similar system successfully tested in medfly. While embryonic lethality is highly preferable to pupal death, for transgenic release use of the two lethal systems together may be advantageous to ensure that reversion or modification of either one does not allow transgenics to survive in the field, that might result in re-population of the targeted species.

Keywords: *Anastrepha*, SIT, conditional lethality, DTS mutations, tet-suppressible lethality

GENETICS ANALYSIS OF *CERATITIS CAPITATA* (WIEDEMANN) POPULATIONS USING MITOCHONDRIAL DNA SEQUENCES.

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Background: The *Ceratitis capitata* species, is, among the Tephritidae family, one of the most damaging pests. From its putative origin in Africa, this species colonized the Mediterranean area and then, expanded all over the world with tropical or temperate climate. The present work reports a molecular phylogenetic study on geographic samples of *Ceratitis capitata* species.

Methods: Flies from six different geographical origins, three from Spain, Mediterranean area, and other three from Africa origin (Kenya, Morocco, and Tunisia), were used for sequencing. A mitochondrial DNA region encompassing 344 bp have been sequenced. The segment include *cytb*, *tRNA^{Ser}* and *ND1* genes. DNA was extracted from individual flies, and amplified with primers CBF1: 5'-ACATGAATTGGAGCTCGACCAGT-3' and NR1: 5'-GGTACATTACCTCGGTTTCGTTATGAT-3', designed by Dr. Fleming (Applied Plant Science, The Queen's University of Belfast, United Kingdom) and based on *Drosophila yakuba* sequences (Clary and Wolstenholme, 1985). Total DNA was extracted from individual flies according to Reyes, Ochando *et al.* (1997). The polymerase chain reaction (PCR) was used to amplify and DNA amplifications were performed in 100 µl reaction volumes containing 4 µl DNA, 10 µl Ecotaq PCR buffer (Ecogen), 2mM MgCl₂, 10 pmol CBF1, 10 pmol NR1, 0.2 mM dNTPs and 2.5 U EcoTaq DNA polymerase (Ecogen). Double-stranded amplified products were purified using the High Pure PCR Product Purification Kit (Boehringer-Manheim) and used as templates for sequencing reactions. These reactions were performed using an ABI PRISM 377 DNA sequencer in the "Servicio de Secuenciación de la Universidad Complutense".

Results: The amplified segments correspond to positions 11523-11867 in the *Drosophila yakuba* mtDNA sequence. All the flies, European and African, showed the same haplotype. The obtained unique sequence was compared with those obtained in previous work and published in the GenBank (<http://www.ncbi.nlm.nih.gov/Genbank>) from *C. cosyra*, *C. rosa*, *B. oleae*, *B. cucurbitae*, *B. dorsalis*, and *B. tryoni* (accession numbers: AY096092, AY096094, AY096087, AY096081, AY096083, AY096088). Sequences were different for different species.

Conclusions: The mitochondrial segment analyzed show a very high conservative sequence. However the time elapsed from colonization and the high density numbers of the populations of *C. capitata*, no variability is detected, and even, surprisingly enough, no variability is detected in African populations. The sequence is useful for taxonomic purposes.

Keywords: *Ceratitis capitata*, mitochondrial sequences, haplotypes.

FEMALE-SPECIFIC EMBRYONIC LETHALITY SYSTEM FOR *CERATITIS CAPITATA* (WIEDEMANN).

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Background: The principle of the Sterile Insect Technique (SIT) involves mass production, mass rearing and mass release of sterile pest insects in the field to control the wild population. SIT is more effective when only sterile males are released and production of only males also reduces the general cost of carrying out an SIT programme. A female-specific lethality system based on a female specific splicing intron was developed for transgenic sexing in *Ceratitidis capitata*, possibly to overcome the fitness problems of classical genetic sexing strains (GSS). However, this transgenic sexing system showed lethality predominantly during the pupa stage. To create an early-acting lethality system, we combined the principle of this female-specific lethality system with an embryonic lethality system to yield a female-specific embryonic lethality system.

Methods: A sexing effector construct driven by the heterologous transactivator tTA and containing a female-specifically spliced intron inserted into the coding region of the proapoptotic gene *hid* was created and inserted into *C. capitata* via *piggyBac*-mediated transposition. Germ-line transformed flies were confirmed by fluorescence microscopy on G1 adults. The resulting transgenic sexing effector lines were crossed with driver lines mediating early embryonic expression of the tetracycline-controlled tTA to generate lethal lines. To assess female-specific embryonic lethality, adults and larvae of these lethal lines were reared on tetracycline-free media and the sex of the subsequent adult progeny checked. Lethality was assessed for both heterozygous and homozygous adult progeny.

Results: Different lethal lines showed varying strengths of female-specific embryonic lethality, with some lines showing complete female-specific lethality. This lethality does not affect male flies in any way observable to us. Female-specific lethality was suppressed on rearing adult flies and their larvae on 10µg/ml tetracycline media. *In situ* hybridizations showed the expected sequential activation of the components of this female-specific embryonic lethality system.

Conclusion: The result of our study establishes female-specific embryonic lethality in the pest insect *C. capitata*, and such a system would also be relatively easy to develop for other tephritid species of agricultural importance. Early-acting female-specific lethality systems should offer cost-effective sexing of pest insect strains once introduced into SIT programmes.

Keywords: *Ceratitidis capitata*, integrated pest management, germ-line transformation, fluorescence microscopy, female-specific embryonic lethality

MOLECULAR TOOLS FOR THE PATHWAY ANALYSIS OF *CERATITIS CAPITATA*.

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Background: Outbreaks of medfly, *Ceratitidis capitata* (Diptera: Tephritidae), occur frequently in the United States. Determining the source of these introductions is key to the eradication of this pest. Unfortunately, morphological characters cannot be used to identify captures to populations-level. However, there are various molecular methods available for population analysis of medfly. These pathway tools are important to the eradication of this pest because they can be used to help develop better quarantine programs, for settling trade disputes, and to help provide valuable information for evaluating eradication programs. The USDA APHIS Mission Lab is assessing the utility of two mitochondrial and twenty-two nuclear markers for their use in the development of a comprehensive set of diagnostic tools for this pest. In order to facilitate the development, we are testing these markers with specimens from worldwide collections of recently-gathered wild and sterile insect technique (SIT) Mediterranean fruit flies.

Methods: In order to evaluate the performance of nuclear and mitochondrial DNA for pathway analysis, we are examining the genetic diversity of numerous Mediterranean fruit fly collections. We are using the polymerase chain reaction and direct sequencing of mitochondrial COI and ND5/ND4 DNA. We are also exploring 22 previously developed microsatellite makers for their use in examining these collections.

Results: Approximately 1900 individual flies from 53 collections and 19 different countries were gathered and submitted to the USDA APHIS Mission Lab fruit fly repository in the spring of 2010. Both COI and ND5/ND4 sequences from the flies were analyzed along with microsatellite loci. Our initial assessment of the mitochondrial DNA sequences suggests that haplotypes maintain a regional specificity. The microsatellite markers we evaluated show an increased resolution as compared to mtDNA and may serve for fine scale population analyses.

Conclusions: Both mitochondrial and nuclear sets of markers provide have a high potential for effective pathway analysis tool development. Further work is needed in order to properly evaluate their utility and develop an effective set of reliable pathway analysis tools.

Keywords: Mediterranean fruit fly, PCR, DNA

PRELIMINARY STUDIES ON THE PRESENCE OF TWO HAPLOTYPES OF THE OLIVE FLY SYMBIONT “*CANDIDATUS ERWINIA DACICOLA*” IN ITALY.

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Background: A very close association between the olive fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) and bacteria has been known for more than a century since Petri firstly described *Pseudomonas savastanoi* as its symbiont. Recently, using molecular techniques, the presence of an hereditary, host specific, unculturable symbiotic bacterium, designated as ‘*Candidatus Erwinia dacicola*’, has been confirmed in all wild specimens even when adults emerged from previously surface sterilized pupae. The present work aims to characterize the possible existence of different haplotypes of “*Ca. E. dacicola*” and their distribution in Italian territory.

Methods: Two-three day-old flies (n= 281), collected from 26 locations across the Italian range of the olive tree, were aseptically dissected to remove the oesophageal bulbs. The DNA of their content was extracted using a salting-out protocol, and an amplification by PCR was performed on the 16s rRNA, using universal bacterial primers. The contents of some olive fly oesophageal bulbs were also cloned using the P-GEM-T Easy vectors (Promega). PCR products and clones, randomly chosen from the constructed libraries, were subjected to DNA sequencing and restriction enzyme detection.

Results: With the exception of a few cases (1.15%) GenBank similarity search showed that the amplified DNA fragment was similar to 16S ribosomal RNA gene of “*Ca. E. dacicola*”. When aligning the sequences obtained two “*Ca. E. dacicola*” haplotypes were found, one being slightly more prevalent than the other (56%). Populations were significantly different from each other in the relative frequencies of the two haplotypes; in particular there seems to be a positive, significant correlation in their distribution along the Tyrrhenian coast that does not occur along the Adriatic coast. Moreover the two haplotypes never co-exist in the same fly, as confirmed by cloning. Populations monitored for three consecutive years show no significant differences in the haplotype frequency.

Conclusions: “*Ca. E. dacicola*” presents at least two haplotypes in Italy whose distribution is not random but significantly different in different regions with an evident gradient of transition, not influenced by sampling in terms of time. Extending the sampling to the Mediterranean area will be the next step in this research.

Keywords: *Bactrocera oleae*, “*Candidatus Erwinia dacicola*”, symbiont, haplotypes.

OLFACTION IN INSECTS: MOLECULAR CHARACTERIZATION AND EXPRESSION PROFILES OF PUTATIVE PHEROMONE-BINDING PROTEIN GENES FROM THE MODEL PEST SPECIES *CERATITIS CAPITATA* (DIPTERA:TEPHRITIDAE).

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Background: In insects, pheromones play an important role in intra- and inter-sex communication, inducing specific behavioural responses in terms of sexual attraction for mate-finding, mating aggregation, or host-marking of oviposition sites. Odour perception is regulated by a fine molecular pathway that involves multigene families including odorant-binding proteins (OBPs) and chemosensory proteins (CSPs). According to the type of ligand, OBPs are classified in general odorant-binding proteins (GOBPs) and pheromone-binding proteins (PBP). Although notable progress in the study of pheromone perception has been achieved in several species, the molecular basis underlying the outstanding selectivity and sensitivity of olfaction in pests such as the Mediterranean fruitfly, *Ceratitis capitata*, is still unknown. In this study we aimed at furnishing the first insights on the pheromone perception process in this species.

Methods: A well established *C. capitata* ISPRA strain reared under standard conditions was used for the experiments. Two different cDNA libraries were investigated by BlastX and tBlastX analyses to identify medfly PBP homologues. The five sequences that showed high similarity to PBP genes of other insect species were first characterized (cloning, 5'-3'RACE-PCR, PCR) and then analyzed to define their expression profiles in relation to sex, tissue, maturation and mating status (RT-PCR, Northern Blot, qPCR). *In silico* analyses were performed to verify the presence of all the features of the PBP family and to obtain a hypothetical 3D protein structure.

Results: We report the cloning, characterization and expression profiles of five putative *PBP* genes (*CcPBPI* to *CcPBP5*) isolated from two cDNA libraries. Molecular analyses revealed the presence of all the features of the PBP family (signal peptide, hydrophobic domain, six conserved cysteines). Gene structures showed high similarity to *Drosophila* putative homologues, except for the presence of longer introns in the medfly. Expression profile analyses showed that i) all the genes were highly over-expressed in head; ii) transcription levels were higher in females than in males, apart from *CcPBP4*; iii) sexually mature males and females showed higher transcript levels of each gene; iv) after mating the transcript levels in females tended to decrease, while males did not experience such a strong effect.

Conclusions: The finding of multiple potential PBPs in the medfly suggests that PBPs may also play a role in the differentiation of pheromone components by differential binding capacities. Future biochemical, functional, behavioural and transgenic assays, will clarify the mechanisms underlying odour and pheromone perception. This knowledge will enable the identification of PBP ligands, to determine their roles in medfly chemoreception and, in the long term, to use pheromone components as target molecules in field studies to develop synthetic attractants or repellents for pest control programs.

EXPRESSED SEQUENCE TAGS (ESTs) IN THE OLIVE FLY, *BACTROCERA OLEAE* (DIPTERA: TEPHRITIDAE).

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Background: The olive fruit fly, *Bactrocera oleae*, is the major pest of the olive tree. Despite its importance, very little genetic and molecular knowledge is available. The present study is a first attempt to identify and partially characterize *B. oleae* expressed sequence tags (ESTs). A few possible uses are also explored.

Methods: Random cDNA clones were isolated from an adult *B. oleae* library and the generated ESTs were annotated through BLASTX similarity searches. A few of them were physically mapped by *in situ* hybridization. Codon usage was determined for the resulting open reading frames. Seventeen Exon-Primed-Intron-Crossing (EPIC) primers were developed and tested on eleven Tephritids.

Results: One hundred and ninety-five randomly selected cDNA clones and the generated ESTs were annotated through BLASTX similarity searches. A set of 159 unique putative transcripts were functionally assigned using Gene Ontology terms in broad categories of biological process, molecular function and cellular component based on *D. melanogaster* matches. Moreover, the cytogenetic location of 35 ESTs by *in situ* hybridization to *B. oleae* polytene chromosomes was defined. The deduced codon usage of the isolated ESTs suggested a conserved pattern of *B. oleae* with its closest relatives. Additionally, the comparative analysis of *B. oleae* ESTs with the homologous *D. melanogaster* genes led to the development of 17 nuclear EPIC-PCR markers for the amplification of intron sequences of 11 Tephritids. Sequencing analysis of several cross-amplified intron sequences revealed a high degree of conservation among *Bactrocera* species and a varying transferability of the generated markers across the examined genera.

Conclusions: The generated *B. oleae* EST collection constitutes the largest set of putative expressed genes in the species. The resulting low resolution EST map more than doubles the available entry points to the insect's genome and can assist syntenic comparisons with other distant species. EPIC analysis can provide a useful tool for the clarification of phylogenetic relationships among different species, particularly in cases of species complexes.

Keywords: Gene Ontology (GO), *in situ* hybridization, EPIC markers, codon usage

Session 4
Risk Assessment, Quarantine &
Post-harvest Treatments

DETECTION OF *ANASTREPHA GRANDIS* (MACQUART) IN CUCURBITS WITH THE USE OF INTEGRATED MEASURES IN A SYSTEMS APPROACH FOR PEST RISK MANAGEMENT IN THE STATE OF GOIÁS, BRAZIL.

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Background: The presence of fruit flies in trade fruit crops, represents an obstacle to the production and marketing of fresh fruits in Brazil as well as worldwide, due to the direct damages caused by the attack of the larvae as well as to the impediments to exportation, in the form of quarantine measures imposed by importer countries. Fruits of the Cucurbitaceae family are hosts of *Anastrepha grandis* (Macquart), which is among the fruit fly species that are subject to quarantine restrictions. Detection survey of *Anastrepha grandis* was concluded to provide information regarding the occurrence of this species. The results might serve as scientific basis so that several municipalities in Goiás State can implement the Integrated Measures in a Systems Approach for Pest Risk Management and ensure the phytosanitary security of Brazil's fruit exportation.

Methods: The monitoring was conducted at production units in the properties registered in the Integrated Measures in a Systems Approach for Pest Risk Management in the municipalities of Carmo do Rio Verde, Cristalina, Goianésia, Ipameri, Itapuranga, Jaraguá, Maurilândia, Rio Verde, Santa Helena, São Miguel do Araguaia and Uruana, on watermelon, melon and pumpkin crops. It used McPhail traps with 5% hydrolyzed corn protein (food attractant), with density of 1 trap/5 hectares and inspection on a weekly basis, within the period of December 2004 to February 2010. After each service, the traps were washed and the attractant was replaced. All the captured insects were transferred to vials containing 70% alcohol, and sent to the Insect Identification Laboratory at the Escola de Agronomia e Engenharia de Alimentos da Universidade Federal de Goiás (UFG) for further identification.

Results: The Index fly/trap/day keep on the acceptable limit in the Brazilian legislation for implementation and maintenance the Systems Approach for Pest Risk Management. Number of specimens of *Anastrepha grandis* (Macquart) detected in 2008 and 2009: Ipameri (01), Cristalina (01), Jaraguá (01), Uruana (03), Rio Verde (01) and Goianésia (07).

Conclusions: The species *Anastrepha grandis* (Macquart) was detected in 2008 and 2009 in the municipalities of Ipameri, Cristalina, Jaraguá, Uruana, Rio Verde and Goianésia, at Goiás State, in Brazil. The results show the low density of *A. grandis* (Macquart) registered in the fields of production of cucurbits throughout the year and in the general population remained within non-detectable levels in the most of the years. Detection survey confirm previous studies which concluded that the pest populations have low density, therefore, they are more important due to quarantine controls, than the damages it actually causes.

Keywords: Cucurbitaceae, fruit fly, *Anastrepha grandis*, Pest Risk Management, detection.

IMPLANTATION OF THE INTEGRATED MEASURES IN A SYSTEMS APPROACH FOR PEST RISK MANAGEMENT IN THE STATE OF GOIÁS, BRAZIL.

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Background: The presence of fruit flies in trade fruit crops represents an obstacle to the production and marketing of fresh fruits in Brazil as well as worldwide, due to the direct damages caused by the attack of the larvae as well as to the impediments to exportation, in the form of quarantine measures imposed by importing countries. Fruits of the Cucurbitaceae family are hosts of *Anastrepha grandis* (Macquart), which is among the fruit fly species that are subject to quarantine restrictions. To export fresh fruits of cucurbits crops to countries devoid of fruit flies, the countries with the presence of fruit flies should have their fruit coming from pest free areas or areas with implementation of the Integrated Management Measures for Risk Management (SMR) officially recognized, ensuring products free of fruit flies. The work aimed at implementing the Integrated Measures in a Systems Approach for Pest Risk Management - RMS for pest *Anastrepha grandis* (Macquart) in the State of Goiás, in order to meet the requirements of importing countries which impose fitossanitary restrictions of cucurbitaceous trade.

Methods: The implantation of the RMS is subject to the detection of the pest *Anastrepha grandis* (Macquart) in the proposal area, according to Instruction Normative 16/2006, which establishes the monitoring for at least six uninterrupted months before the request for official recognition. The Farms of properties inscribed on watermelon, melon and pumpkin cultivation, were registered in the State Board of Plant Protection Plant and monitoring *Anastrepha grandis* (Macquart) within the period of December 2004 up to June 2005, from April to September 2007 and from April to September 2008. The farms were audited and recognized by the Plant Protection Division of Ministry of Agriculture, Livestock and Food Supply through the Normative Instruction 41/2006, 22/2008 and Resolution 1/ 2009.

Results: The state of Goiás in Brazil, implemented and obtained official recognition by the Ministry of Agriculture, Livestock and Food Supply the Mitigation Risk System -RMS for pest *Anastrepha grandis* (Macquart) in 2006, 2008 and 2009 in the municipalities of Carmo do Rio Verde, Cristalina, Ipameri, Itapuranga, Jaraguá, Maurilândia, Rio Verde, Santa Helena and Uruana.

Conclusions: The implantation of SMR has allowed, since 2006, the exportation of watermelon, melon, pumpkin fresh fruits which led to the expansion of trade and the opening of new markets for the State of Goiás.

Keywords: Cucurbitaceae, fruit flies, Integrated Measures in a Systems Approach for Pest Risk Management, *Anastrepha grandis*, exportation

WHEN FRUIT FLIES FLY. DATA ON THREE YEARS OF TEPHRITIDAE INTERCEPTION BY THE FRENCH NPPO IN ROISSY CHARLES-DE-GAULLE AIRPORT.

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Background: All non european Tephritidae are in quarantine pest list for EU area. In Roissy Charles de Gaulle airport, the NPPO inspectors control commodities (fresh fruits/ vegetables) that come from different parts of the world. Main pathways, commodities and fruit flies groups are presented.

Methods: The inspectors are formed to recognized Tephritidae larva and other quarantine pests. When they found larvae, several are send to LNPV* for confirmation allowing official notification. If possible, the other available larvae are reared and resulting adults are sent to LNPV for species identification. Presented datas correspond to the results of these rearings during the years 2007 to 2009.

Results: Over the followed period 716 rearings gave adults identified to species (5433 specimens). Four genus are represented, by order of importance: *Bactrocera*, *Ceratitis*, *Anastrepha* and *Dacus*. Fruits Flies were taken on fourteen varieties of fresh fruits/vegetables. The main is *Mangifera indica*, followed by *Annona* spp., *Capsicum* spp., *Syzygium* spp. and *Psidium guajava*. The geographical origin is mainly Asia, then Africa and to a lesser extent neotropical area.

Conclusion: Data collecting during last there years allow identifying possible pathways of introduction of the fruit flies in EU. Only species of agronomic importance are recorded pointing out a strong link between the species and its host-plant (ex: *Bactrocera invadens* and mango) or between the species and the area of origin (ex: *Anastrepha* spp. and neotropical area). The observation of *Bactrocera invadens* in the records shows the importance of the survey and the interest of the identification at species level in following pathways.

Keys words: quarantine, pathway, interception

FRUIT FLY MANAGEMENT BEGINS AT HOME.

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Background: Three species of fruit flies of economic importance occur in South Africa - Mediterranean fruit fly (*Ceratitidis capitata*), Natal fruit fly (*C. rosa*) and marula fruit fly (*C. cosyra*). Their pest status is exacerbated by the fact that they are international quarantine pests, restricting the free trade of export fruit. Chemical options for fruit fly control are limited to baits comprising an attractant and an insecticide, and the use of "attract and kill" technology (bait stations). The sterile insect technique is also used in certain areas against *C. capitata*. None of these strategies is regarded as a 'stand-alone' - all possible strategies should be incorporated in an integrated management approach also involving vineyard/orchard sanitation and host plant management. Success with fruit fly control in South Africa varies; poor control is mostly due to non-compliance with recommendations, especially sanitation and host plant management. Understanding fruit fly behaviour, and in particular their migration behaviour and easy adaptation to home garden environments, can play a major role in limiting infestation in commercial plantings. In this regard a trial was carried out to monitor the occurrence of *C. capitata* and *C. rosa* simultaneously in a block of commercial table grape vineyards and in nearby home gardens over two seasons, to identify the time and place during the season when fruit fly populations are most vulnerable to management interventions, and to identify periods in the season and target areas for optimal fruit fly control.

Materials and Methods: A vineyard of five adjacent blocks of export table grape (± 12 ha) with reportedly high populations of fruit flies were monitored from December 2006 to October 2008 on a farm in the Worcester district of the Western Cape. Two home gardens in a residential area approximately 400 m from the vineyard site were simultaneously monitored. Five Chempac® traps baited with 3-component lure were placed in the table grape vineyard, and one each in suitable shade trees in the two home gardens, and serviced monthly. All male and female fruit flies were recorded. The vineyards were harvested from January to April.

Results: In the vineyard blocks, very high fruit fly populations occurred from March to June, while fruit flies were absent or in very low numbers from July to February. In the residential gardens, moderate but fluctuating populations occurred throughout the year, including July to February when fruit flies were absent in the vineyards.

Conclusions: The results highlight the role urban areas play in fruit fly infestation levels in commercial fruit. Adults leave commercial fruit plantings in early winter, seeking shelter, food and breeding sites in residential gardens with host plants year-round. They re-infest commercial vineyards and orchards in summer when ripe fruit is available and susceptible. The greatest control effort should therefore be made in all gardens from early winter to early summer to limit the size of populations that will later migrate to vineyards and orchards in summer. All possible strategies should be used: fruit fly bait, bait stations, host plant management, sanitation, and SIT where applicable.

Keywords: Monitoring, migration, commercial fruit, home gardens, management strategy

THE POTENTIAL GEOGRAPHIC DISTRIBUTION OF *BACTROCERA ZONATA* (SAUNDERS) IN EUROPE AND THE MEDITERRANEAN BASIN.

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Background: Ten years ago, the peach fruit fly (PFF) *Bactrocera zonata* (Saunders) seemed to present a lesser risk for Europe than other fruit flies as its geographic distribution was restricted to tropical areas. However its rapid spread and establishment in Egypt has confirmed that the PFF should be treated as a priority, at least, by Mediterranean countries. In this article we have developed a predicted distribution of the PFF for Europe and the Mediterranean Basin using CLIMEX modelling package.

Methods: Climate matching software CLIMEX was used to synthesize available information from diverse sources and assess the invasion potential of the PFF within Europe and the Mediterranean Basin. A Composite Match Index (CMI) was determined with the 'Match Climates' function in order to match: (a) the ranges in Egypt where the fly is introduced with its native range in India and Pakistan; (b) the ranges in Europe and the Mediterranean Basin with the current distribution of the PFF. The Ecoclimatic Index (EI) was estimated in order to have an overall measure of favourableness of each European location for permanent occupation by the PFF.

Results: Broadly speaking, the climate of India is not similar to that of Egypt. On the other hand, the climate of Pakistan shows significant similarities with most of the Egyptian locations. The climatic similarity between Egyptian and the European locations is variable: Alexandria and El Tur, where the PFF is known to occur, are similar to several locations of Spain, Greece, Italy and Portugal but the climate of El Cairo is not comparable to any location in Europe. Regarding the EI, more than 40 locations within Europe have obtained an EI > 30, which means that their ecoclimatic conditions are very favourable for the pest establishment. In these places the PFF is predicted to have an economic impact.

Conclusions: The locations within the areas of origin of the PFF are not climatically similar. This may lead to the contention that the Indian populations of the PFF have different climatic requirements to the populations found in Pakistan or those introduced in Egypt and the Near East. The climate model supports that Mediterranean and semi-arid climates are predicted to be suitable for the long-term survival of the PFF. Due to the predicted capacity of PFF to withstand drier climatic conditions than those traditionally associated to this fly, much of the Mediterranean Basin could be at risk of invasion.

Keywords: Climate matching, Pest risk prediction, CLIMEX, invasion potential, climatic conditions.

DETERMINATION OF THE MOMENT UNDER WHICH VERNA LEMON (*CITRUS LEMON*) IS SUSCEPTIBLE TO *CERATITIS CAPITATA* IN THE SPANISH SOUTHEAST.

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Background: Until 2009, the lemon was not included in the list of quarantine host of *Ceratitidis capitata*, although there was evidence of isolated incidents of infested fruits. Therefore special regulatory rules for international trade were not established. In 2006, at the end of the export campaign to US, fruits were found infested on arrival from a batch of “Verna” lemons from the Spanish mainland southeast. The singular climatic and agronomic conditions of the 2006 campaign brought about harvesting of fruits damaged or senescent which were infested. In order to adjust the conditions of harvesting of fruits for export, it was needed to determine the susceptibility time frame and the conditions which allows the complete development of *C. capitata*.

Methods: Over the last two campaigns Verna lemons have been regularly sampled from three orchards registered in the official record for the export of fruits to US. Additionally, *C. capitata* monitoring was established for the three orchards along the study period. Fruit sampling started at fruit colour change until early July. Commercial size fruits were taken each week during the first month and every 3 weeks since the fruits were completely yellow. For each orchards and group of 100 fruits it was determined: a) Natural infestation, keeping them in incubation for 90 days at 25°C, between 70 and 100% relative humidity and a photoperiod of 14:10 hours light:dark. b) Fruit susceptibility by artificial infestation (by exposing the collected fruits to a population of *C. capitata*, fruits were incubated under the same conditions).

Results: Natural infestation in the orchard occurs at a time when the levels of capture of adults in the plots are highest. Shortly before detection of natural infestation, a small proportion of fruits were susceptible to artificial infestation. In controlled conditions *C. capitata* was able to infest and complete development in those yellow Verna lemons.

Conclusions: The results show that natural infestation of Verna lemons depends on the characteristics of the fruits (colour and damage) and on the adult immigrants populations that arrive to the plots from other host plants.

Keywords: *Ceratitidis capitata*, quarantine, susceptibility.

INDICATOR PARAMETERS OF SUSCEPTIBILITY OF VERNA LEMONS (CITRUS LEMON) TO *CERATITIS CAPITATA* (WIEDEMANN).

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Background: Is abundant the bibliography which report contamination episodes by *Ceratitis capitata* of certain varieties of lemons when these are overripe. In the summer of 2006 adults of this species were found in an export batch of “Verna” lemon from Spanish southeast to US. Following this event were initiated studies to try to determine some characteristic of the fruit, to distinguish the susceptible fruits that can be infested of non-susceptible, in order to facilitate regulatory inspections in the marketing.

Methods: Over the last two campaigns have been harvested “Verna” lemon fruits in orchards of the three most important production areas in the Region of Murcia, from the months of November to June-July. The samples were collected each week during the colour change and every 3 weeks later. Some of the fruits were used to determine the natural infestation and to evaluate the susceptibility from exposure to adult populations of *Ceratitis capitata* in artificial conditions. Another part was used to determine the following parameters: acidity of the juice, °Brix, colour, peel thickness, equatorial diameter, hardness of the peel, chemical composition of the peel (albedo and flavedo), etc. The correlations between these parameters have been studied and the infestation in the different sampling dates.

Results: Not has been found a representative parameter of the characteristics of the fruit that is associated with the susceptibility or resistance to infestation by *Ceratitis*. The parameter more indicative of the susceptibility is the ratio between the force required to break the peel and the distance at which rupture occurs. Also, the composition of the albedo would be indicative of the susceptibility or resistance, because the larvae did not complete their postembryonic development, when feed on albedo

Conclusions: Any parameter representative of the fruit that is closely related to the susceptibility or resistance of fruit infestation by *Ceratitis* has been found. We need to study more thoroughly the ratio between the force required to break the peel and the distance at which rupture occurs, as well as the chemical composition of albedo.

Keywords: fruit fly, export protocol, susceptibility.

CONTRIBUTION FOR DEVELOPMENT OF A *CERATITIS CAPITATA* (WIEDEMANN) RISK MODEL IN TERCEIRA ISLAND.

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Background: The Medfly (*Ceratitidis capitata* Wied.) (Díptera: Tephritidae) worldwide biological adaptability as well as the careless fruit transportation, sometimes infested with eggs or even larvae, were two the most important factors which allowed the fast dispersion of this pest over many continental areas and islands. The behavioral study, as well as the fight and prevention studies about this insect has become very important, because there has been a bigger occurrence of this pest over several fruit cultures, carrying sometimes severe economic impacts to the fruit producers. In the Azores Islands, especially on Terceira Island the behavioral study of this insect has been done by Interfruta and Interfruta II Investigation Projects. Their studies have been covering the population dynamics, dispersion, fruit infestation and some control measures. This work will try to expand the horizons of these studies with the creation of a model about risk prediction of *C. capitata* for Terceira Island.

Methods: For model development, traps capture records from some works done in Interfruta II project are going to be used to try to establish a reasonable multi-correlation ratio between these records and variables such as climatic, soil occupation and fruit availability.

Keywords: Medfly, Risk model, Interfruta projects

THE BANANA FLY, *BACTROCERA MUSAE* (TRYON): IS IT A BANANA PEST?

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Background: The Pest Risk Analysis (PRA) is a central plank of international phytosanitary regulations. A fundamental aspect of PRAs is knowing the risk posed by different pests. Unfortunately, for many crops susceptible to fruit flies, PRAs are often based on generic fruit fly host lists, in which fruit varietal differences, ripening stages and field usage of hosts is rarely, if ever, considered. In Papua New Guinea (PNG), a potential exporter of the commercial banana varieties Cavendish and Lady Finger, the endemic banana fly, *Bactrocera musae* (Tryon), is considered a major pest. As part of developing a PRA for PNG bananas, we studied the abundance, distribution, host use and pest status of *B. musae*.

Methods: Distribution and abundance data for *B. musae* in PNG was generated through an intensive trapping program. Abundance data was correlated with rainfall, altitude and the importance of banana in the local cropping system. Host preference and larval performance of *B. musae* on the commercial varieties “Cavendish” and “Lady Finger” were examined in the laboratory. Field infestation of Cavendish in PNG was examined through a national fruit survey covering four agricultural production areas and bananas picked at the mature green stage of ripeness.

Results: Banana fly was found to be common and widespread throughout PNG. Abundance was not significantly correlated with rainfall ($R^2 = -0.02$, $p = 0.28$) or altitude ($R^2 = -0.01$, $p = 0.35$), but was weakly positively correlated with banana’s importance in the local cropping system (when presented as a percentage of local crop grown) ($R^2 = 0.08$, $p = 0.02$). Despite the fly’s high field abundance, Cavendish bananas picked from unmanaged plots around PNG were essentially unaffected by banana fly. From 111 samples, constituting 381kg of fruit and 2,700 fingers, only 11 fingers were infested (0.41% infestation). Eighty-seven percent of flies reared ($n = 245$) came from only three fingers (0.1% of sample). Laboratory studies reflected field data, particularly for adult oviposition trials: while there was 20% adult emergence from Cavendish and Lady Finger fruit inoculated with *B. musae* eggs, very few flies laid eggs into fruit of either variety.

Conclusions: Under laboratory conditions, the commercial banana varieties Cavendish and Lady Finger are infrequent hosts of *B. musae*. Cavendish is also a rare field host and we suspect the same is true for Lady Finger. In PNG, banana fly infests over-ripe bananas, as well as some sweet native varieties (authors’ unpublished data), but not the main commercial varieties when picked at the mature green stage. This project reinforces the importance of detailed host use studies for developing PRAs, rather than relying on generic literature records.

Keywords: Pest risk analysis; agricultural production, abundance and distribution, host use.

INSPECTION OF ORGANIC PRODUCTS BY DETECTOR DOGS.

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Background: The Incan Trehua is a Program developed by FunBaPa, Iscamen and Senasa. This program is aimed at training dogs for the detection of animal and vegetable derived products. These banned substances should not enter to the protect product areas due to sanitary reasons. The Incan Trehua program means “protector dogs” in Mapuche language and it`s pioneering in Latin America. The aim of this program is to enhance the performance of the Quarantine Patagonian System. By use of the olfactory sense to detect loads with restrictic admissions products. Incan Trehua improves the effectiveness in the sanitary control stations and making the inspectors work easier.

Methods: Beagle and Labrador retriever are the chosen dog breeds for this project. The training period last a minimum of six weeks, during that it stimulates the Memory of Olfactory Fields of the dog. This is achieved trough sterilized lints impregnated in contact with differents fruits and vegetables products and associated to many acts that the dog does, what its called “Associative Kits”

Results & Conclusions: The Incan Trehua program extends its services throughout the most importans land points of control, spread over the provinces of Buenos Aires, Neuquen, La Pampa, Rio Negro and Mendoza, the Neuquen International Airport and the Viedma National Airport, protecting the entry of banned fruit, vegetables and animal origin products to Patagonia. Throughout the six years the program has, we achieved and statistic of, in a hundred vehicles controled by dogs, they detect banned organic products in ten of them. This takes over the statistics of man detection and provide a more friendly and accepted way of search as well.

THE MEDFLY, *CERATITIS CAPITATA* (WIEDEMANN), AS VECTOR OF *ERWINIA AMYLOVORA*, THE CAUSAL AGENT OF FIRE BLIGHT IN ROSACEOUS PLANTS.

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Background: The bacterium *Erwinia amylovora* is the causal agent of fire blight in rosaceous plants, and is one of the most important pests on the EPPO A2 list, being under quarantine measures for plant movement. It has been widely accepted that fruits from fireblight affected countries present an insignificant risk of transmission in fire blight free countries, despite the recent description of the viable but non-culturable (VBNC) condition, a bacterial physiological state that is undetectable by cultural methods. Till this moment, only sap-sucking insects or pollinators had been implied in fire-blight transmission. The Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann) (Diptera: Tephritidae), is a devastating fruit pest worldwide. Besides the direct damage caused to fruit production and export, the medfly also poses a serious indirect threat to the plant health by acting as plant pathogen vector. This work describes the *E. amylovora* transmission ability of medfly and its implication on fruit trade.

Methods: Five days old individuals of IVIA2002 strain were used to determine: i) its transmission ability and ii) the survival of *E. amylovora*. For the transmission ability, medflies were allowed to feed on contaminated mature apples for 48h and then other healthy apples or pear sprouts were challenged to detect transmission during 5-7days. For *E. amylovora* survival assays, medflies were put in contact with contaminated mature apples for 48h, and then tested for *E. amylovora* presence at 7, 14, 21 and 28 days after removing apples. The detection of the plant pathogen was determined in bacterial CCT medium and by PCR, both from plant material and medflies.

Results: The medfly was able to transmit *E. amylovora* to fruit peel or pulp after 5 days exposition at 10^6 - 10^3 cfu, respectively. The medfly induced fireblight symptoms in pear sprouts after 5-7 days of transmission. We were able to isolate culturable *E. amylovora* cells from medfly after 7 or 14 days from exposition (10^3 - 10^6 cfu/medfly). However, with longer bacterium-medfly contact periods, the bacterium adopted the VBNC state. Medfly extracts (21 or 28 days old) containing these VBNC cells, induced fireblight symptoms in fruits and pear sprouts and tested positive by PCR..

Conclusions: This is the first time that the medfly is assessed as a vector of a plant pathogen such as *E. amylovora*. In this host the bacterium maintains its pathogenic potential during almost one month, being transmitted in high numbers to healthy plant material.

Keywords: Medfly, *Erwinia amylovora*, fire blight, transmission, survival, plant pathogen vector.

GEOGRAPHICAL DISTRIBUTION OF *ANASTREPHA GRANDIS* (MACQUART) IN THE STATE OF SÃO PAULO, BRAZIL.

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Background: *Anastrepha grandis* is considered a fruit fly species of quarantine importance and it has been registered in the south, southeast and central of Brazil. In the State of São Paulo is concentrated the highest volume of cucurbitaceae trade for Brazilian market. The objective of the present study was to investigate the geographical distribution of *A. grandis* in the State of São Paulo.

Methods: The occurrence of *A. grandis* was based on the literature, such as theses, articles and reports from the congress. Additional informations were based on field surveys conducted from 2009 to 2010. In the both cases, we considered collections of *A. grandis* from fruit hosts and monitoring studies in many crops.

Results: *A. grandis* is reported in 22 municipalities of northeast, central, southwest and east of the State of São Paulo. Eleven municipalities are located in the east region.

Conclusions: No specimen of *A. grandis* was collected from northwest and extreme west regions of the State of São Paulo, which it was included the squash production areas for exportation.

Keywords: South American Cucurbit Fruit Fly, occurrence, localities, quarantine pest, Cucurbitaceae

THE THREAT OF *BACTROCERA INVADENS* DREW TSURUTA & WHITE, TO SOUTH AFRICA: SURVEILLANCE, CONTINGENCY AND TRADE RESPONSE.

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Background: Exotic fruit flies remain a major concern to South African fruit producers aiming to reach the international market. Not only do exotic flies pose a risk to fruit production, but there is a real threat that export markets may be lost or at least that exports could be temporarily suspended should new species be introduced to the country. *Bactrocera invadens*, an invasive fruit fly of Asian origin, was first detected in Africa in 2003 in Kenya. Thereafter, the fly was reported in several other African countries including those in southern Africa such as Zambia, Mozambique and Namibia. *B. invadens* males are strongly attracted to methyl eugenol which can be used for monitoring of this fly. Since 2006, South Africa has been conducting a fruit fly surveillance programme through collaboration between the National Plant Protection Organisation (NPPO), fruit industries and research organizations for early detection of exotic fruit flies including *B. invadens*. Contingency plans specific to *B. invadens* have also been put in place in South Africa and these include the setting up of a national steering committee on *B. invadens* and the drafting of an action plan specific to the Invader fly where response actions for initial incursions and longer term management strategies are described. Import conditions for fresh fruit have been revised, which has had an impact on trade of certain host commodities from countries where *B. invadens* is present.

Methods: The surveillance network for detection of *B. invadens* currently consists of 616 methyl eugenol baited traps and 136 BioLure 3 component baited traps. Traps are deployed in major towns, ports, airports, high risk border areas as well as in production areas. Traps are serviced at least once a month. The trapping information from the NPPO and fruit industries is then captured in a centralized database.

Results & conclusions: To date, South Africa remains free from *B. invadens*. Should there be an incursion of *B. invadens* in South Africa, a stockpile of emergency materials for use according to the relevant action plan, will be available immediately for eradicating this pest.

Keywords: Invader fruit fly, detection, action plan, trade implications

PATAGONIAN QUARANTINE SYSTEM.

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Background: The Patagonian Protected Region, which is the southernmost area in South America, covers an area of 835.000 km². Its natural boundaries are the Andean Range to the west, the Atlantic Ocean to the east and the Colorado and Barrancas Rivers, along which the terrestrial control posts in every way-in to the protected area are settled, to the north. The Patagonian Quarantine System is conducted and administrated by the Patagonian Zoophytosanitary Barrier Foundation (FUNBAPA), a non governmental organization where institutions coming from both the public and private sectors, like SENASA, regional provinces administrations, and growers and breeders associations, take part. It is a joint organizational model of public and private sectors, non-profit and with an active participation of its components. The aim of the Patagonian Quarantine System is the supervision of the way-in and way-out of plant or animal originated products by the enforcement of federal sanitary regulations in order to avoid the introduction of pests and/or diseases that could affect the local production. Legislation applied through the Patagonian Quarantine System is federal and is originated both in the Ministry of agriculture, livestock and fisheries of the nation (MAGyP) and the Agroalimentary Quality and Health National Service (SENASA).-

Methods: The Patagonian Quarantine System involves 34 terrestrial inspection posts 13 airports, 1 railroad control post and all the seaports of the Patagonian coast. Terrestrial posts work all the year round, 24 hours a day, and in each post the number of inspectors is directly related to the number of vehicles that pass along. More than three hundred people among professionals, technicians and inspectors are involved, being trained and evaluated on an ongoing basis. Supervision and quality control is carried out by the own managers staff and the audits are carried out by SENASA. Inspection work is complemented by the FUNBAPA Incan Trehua Program which uses dogs specially trained for the detection of organic products. Besides, special equipment for the same purpose is used at airports so as to improve and facilitate inspection tasks. The financing of the Patagonian Quarantine System is given by the funds originating from the tariff control, inspection and disinsectionation is levied in terrestrial barrier checkpoints, contributions from the Government and the provinces.

POTENTIAL DISTRIBUTION OF *CERATITIS ANONAE* IN CHINA BASED ON PREDICTION OF ECOLOGICAL NICHE MODELS.

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Backgrounds: The *Ceratitis anonae* (Graham) is native to the Afrotropical region. It is a polyphagous species with wide host ranges including almond, annonaceous, avocado, cherry, coffee, guava, mango, strawberry, and so on. This insect pest is an important quarantine invasive pest for China and its larva are continually intercepted from fruits carried by incoming passengers at Guangdong ports. The potential geographic distribution of this species in China is of considerable concern in terms of biosecurity.

Methods: In this study, three ecological modeling methods (ENFA: ecological niche factor analysis, Mahalanobis Typicality, and Maxent: maximum entropy niche-based modeling) were used to predict its potential distribution. Native range of *C. anonae* was derived from the Royal Museum for Central Africa and the Global Biodiversity Information Facility. Records of occurrence were obtained from the literature and electronic resources. In total, 144 unique records were obtained for *C. anonae*.

Results: The results show that Maxent has the best prediction performance, followed by Mahalanobis Typicality, and ENFA. The difference of prediction capabilities between Maxent and Mahalanobis Typicality is not significant. Based on prediction outcome of Maxent, suitable areas of *C. anonae* in China are limited in Guangxi, Guangdong, Hainan, and a few areas of Yunnan. The probability of *C. anonae* permanently establishing in southern China exist indeed but small. Jackknife analysis indicates that six variables have notable influences on the distribution pattern of *C. anonae*, i.e. ground frost frequency, annual mean precipitation, precipitation of October, precipitation of April, annual minimum temperature, and vapor pressure. More researches on the influence of environmental variables on survival of *C. anonae* are needed to determine its suitable distribution area.

Conclusions: The probability of *C. anonae* permanently establishing in southern China exist indeed but small. Six environmental variables have notable influences on the distribution prediction of *C. anonae*, and further researches about their influence on survival of *C. anonae* are needed.

Keywords: *Ceratitis anonae*; ENFA; Mahalanobis Typicality; Maxent; potential distribution prediction

Session 5
SIT Principles & Application

OLIVE FLY: FROM SMALL SCALE PRODUCTION TO MASS-REARING.

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Background: The olive fly, *Bactrocera oleae* (Diptera: Tephritidae), is considered an ideal candidate for control by the sterile insect technique (SIT) as part of an integrated pest management approach because it attacks only olives and does not disperse naturally great distances unlike other *Bactrocera* spp. One major constraint in the development of a successful and cost-effective SIT programme for olive fly is the large scale production of high quality mass-reared flies. The aim of this work was to develop cost-effective methods for mass-rearing.

Methods: The following three basic parameters in mass-rearing were examined: Cage density of adult flies, size and design of the adult oviposition cage, and egg collection methods.

Results: The results showed that an adult fly density of 4.1 cm² (internal surface area of cage) per fly in a medium-sized cage (0.036m³) produced up to 11.5 eggs/female/day over the life of flies. Further research on other cage sizes ranging from 0.015 m³ to 0.4 m³ resulted in egg volumes from only 2.8 to 6.8 eggs/female/day. Our newly developed method of egg collection using a flat eggging panel proved cost-effective, more efficient, and enabled us to elevate the colony size to a level that mass-production can be started.

Conclusions: As a consequence of this work, we are now rearing the flies in large cages (0.4 m³) formerly used for mass rearing of Mediterranean fruit fly and we are now optimizing the density of olive fly in cages that are more acceptable for cost-effective mass rearing of this insect.

Keywords: Olive fly, fly density, cage design, egg production

STERILE INSECT TECHNIQUE FOR CONTROLLING CUCURBITS FRUIT FLY, *DACUS CILIATUS* (LOEW) (DIPTERA:TEPHRITIDAE).

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Background: The cucurbit fruit fly, *Dacus ciliatus* (Loew) is one of the most important fruit flies on cucurbits in Iraq. The Iraqi scientist thinking to apply sterile insect technique to control this pest instead of insecticide for environment concern, after they carried out several studies regarding its biology, ecology and behavior.

Methods: Different doses of irradiation were tested on 4-5 days old pupa. Egg laid was measured for each dose, using also irradiated females as control.

Results: The results of this study revealed that the dose of 0.75 kGy caused complete sterility to the females and the dose of 0.90 kGy caused complete sterility to the males when they irradiated as 4-5 days old pupa. The oviposition was recorded in the mating between unirradiated males x irradiated females (1); irradiated males x unirradiated females (2) and irradiated males x irradiated females (3) for the doses 0.15-60 kGy, while for the doses 0.75 & 0.90 kGy no eggs laid were observed for the mating 1 & 3 only. Furthermore, the percentage of eggs hatch was zero for doses 0.75 & 0.90 kGy for the mating 1 & 3, while it was zero for mating 2 for the dose 0.90 kGy only.

Conclusions: The results of this study also showed that doses of gamma rays have no effect on pupation percentage of the larvae produced from hatched eggs of irradiated males & females as pupa of five days with all doses & mated to opposite unirradiated sex or mated together. Finally the results of this study also showed that all doses have no effect on adult emergence percentage & sex ratio.

Keywords: *Dacus ciliatus* , Gamma Rays, Fecundity, Cucurbits

IMPROVEMENT IN PRODUCTION AND QUALITY PARAMETERS AT NEW SIT AFRICA MEDFLY, *CERATITIS CAPITATA* (WIEDEMANN), REARING FACILITY.

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Background: The South African Medfly SIT programme was initiated in 1999 when mass rearing of the *tsl* strain Vienna 7 was started in a small, renovated building in Stellenbosch. A number of strain changes have since taken place, and Vienna 8 has been used since 2003. In 1999 the maximum production capacity was approximately 8 million sterile males per week. Production and quality was adversely affected by factors including equipment breakdown, inadequate environmental insulation, lack of space, and micro-organism growth in breeding rooms, all of which was compromised by a shortage of funding. The building was old and the general infrastructure was increasingly becoming degraded. In 2001 a new Chromodec® insulated-panel building was erected to house the adults and a quality control laboratory. Although this led to more stable production and quality, larval rearing and pupal handling in the old building, and ultimately sufficient production of high quality sterile males, remained at risk from the failing infrastructure. Roll-out of the SIT programme to new areas required an increase in production, which put further pressure on the facility. A new building to house the immature stage rearing and handling was essential.

Development: In 2010, following a considerable injection of funding from government, a 480 m² Chromodec® insulated-panel extension was added to the adult room for immature stage rearing. The capacity of this new facility is estimated to be at least 40 million sterile males per week, which will allow a far greater fruit production area to be included under the SIT blanket.

Results: In the 6 months since the new building was commissioned, production has significantly stabilised, and key production and quality parameters have all increased, in some cases substantially, in comparison with the previous 12 month-period in the old building. Overall average increases in the filter, amp 1, amp 2 and release streams (in percentage points) were as follows: egg production, 40.9; efficacy, 25.7; egg hatch (48 h), 4.0; flight ability (colony females), 3.6; flight ability (colony males), 3.9. Pupal weight in the release stream increased by 2.5 percentage points, and flight ability of sterile males by 2.5. The long-term trends in all parameters from the new building show a narrower band of fluctuation than in the old building, and all daily and weekly figures are mostly on or above target. Currently, 11 million sterile males are released weekly in three separate production areas, protecting a total area of nearly 24,000 ha of fruit.

Conclusions: The above improvements in production and quality augur well for the planned expansion of Medfly SIT in the Western Cape and even further afield in the near future, and for the Medfly SIT programme in South Africa in general.

Keywords: Medfly, mass-rearing, *tsl*, improved quality, SIT

SUITABLE LARVAL DIETS FOR *ANASTREPHA FRATERCULUS* (DIPTERA: TEPHRITIDAE).

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Background: The genus *Anastrepha* (Diptera:Tephritidae) is the largest and most economically important genus of true fruit flies in the American tropics and subtropics. It is indigenous to the Americas, presently with no distribution outside the Western Hemisphere. Among other species, the South American fruit fly, *Anastrepha fraterculus* (Wiedemann, 1830) is highly economic important in South America, especially in Brazil. Attempts to improve diet quality for mass rearing *A. fraterculus* have been ongoing for more than twenty years. The diets currently used throughout the world were based on combined ingredients taken in account their availability, physical properties and economics with little emphasis on nutrient value. A mass rearing system and Sterile Male Technique (SIT) for *A. fraterculus* are underway and will remain a challenge. A technique to mass rear *A. fraterculus* is the start point for the development of SIT. The objective of the present work was to identify appropriate ingredients for suitable larval and adult diets to be used as a platform to develop a protocol for mass rearing the South American fruit fly.

Methods: A screening of eleven different larval diets (four replications) was performed in order to find suitable and economic diets for further comparison tests. The efficacy of the diets was determined by statistical analysis including egg hatching, pupal recovery, pupae weigh, adult emergence and adult flying ability. The general procedure for quality control tests were based on protocols specified in the International Fruit Fly Control Manual (FAO/IAEA/USDA, 1998).

Results: The final goal of a fruit fly mass rearing production system is a consistent result of healthy and competitive adults. This success is very dependable of high control quality of all laboratory procedures and specially a suitable and economic diet for larvae. From the results of these larval diets experiments, there is clear evidence that the source of protein is determinant for getting healthy and competitive adults. Showed by fly ability test. From six diets tested, three of them with 9.0, 10.0 and 13.0% of protein and 20.0% of cane bagasse as the bulk agent presented the best results for egg hatching, pupal recovery, pupal weight, adult emergence and fly ability when is compared with the other diets with less protein content. Since there were no statistical differences among these three diets it is clear that is more economically feasible to recommend the diet with 9% of protein.

Conclusions: It is clear that protein in diet larvae improves larval development, pupal recovery and adult fly ability. Larval diets with 9.0% of protein show to be adequate for a mass rearing program with focus on SIT. The bulk agent sugar cane bagasse improves larval feeding by facilitating the larvae movement though out the diet mass.

Keywords: South American fruit fly, SIT, pupae, mating, fly ability

INFLUENCE OF PRERELEASE DIET TO *BACTROCERA DORSALIS* (HENDEL) AND *BACTROCERA CORRECTA* (BEZZI) STERILE MALES ON MATING PAIRS.

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Background: Released sterile males fed only water-agar are currently applied in fruit fly control project area in Thailand. In general, effectiveness of protein was shown to the sexual maturity in fruit fly males for the mating capability. We reported here on influence of prerelease diet of sterile males on mating pairs which indicate their successful of mating performance.

Methods: Sexing separation of the 53th generations of *Bactrocera dorsalis* (Hendel) and the 55th generations of *Bactrocera correcta* (Bezzi) sterile mass reared males were conducted within 24 hours of eclosion. Five groups of neonate fruit flies were fed with water-agar only, sugar (S), sugar and protein hydrolysate ratio 3:1 w/w (SP = 3:1), sugar and protein hydrolysate ratio 2:1 w/w (SP = 2:1) and protein hydrosate (P) only for two days. Then each group were given a standard adult diet SP (3:1) and water throughout the experiment. During 15 testing days of *B. dorsalis*, each 2+n day-old (n=1-15) of 50 sterile males fed with difference type of diet were mated with 50 virgin mass reared females, 12 day-old. With the same procedure during 18 testing days of *B. correcta*, each 2+n day-old (n=1-18) of 50 sterile males fed with difference type of diet were mated with 50 virgin mass reared females, 17 day-old.

Results: From the 7th-15th testing days after prerelease diet feeding, young sterile *B. dorsalis* and *B. correcta* fed with S, SP = 3:1, SP = 2:1 and protein hydrolysate showed higher different mating percentage than those fed only water-agar significantly. Total testing period (15 days for *B. dorsalis* and 18 days for *B. correcta*) percentage of mating pairs of sterile male fed diet formula were highly significant difference than other fed with water-agar only. Young sterile male of *B. dorsalis* fed protein hydrolysate showed the highest percentage of mating pairs but not significantly difference to those fed S, SP = 3:1; and SP = 2:1. *Bactrocera correcta* fed with SP=3:1 achieved the highest percentage of mating pairs and significantly higher than sterile males fed with S and P only but not significantly difference to sterile males fed SP = 2:1.

Conclusions: Prerelease diet, sugar only, combination of sugar and protein hydrolysate 3:1, 2:1 and portein hydrolysate only showed non significantly difference of mating pairs for sterile *B. dorsalis* males but *B. correcta* males fed with SP=3:1 and SP=2:1 showed mating percentage significantly superior than S and P feeding. Sterile males of *B. dorsalis* and *B. correcta* fed only water-agar as prerelease diet obtained very low mating success.

Keywords: *Bactrocera dorsalis*, *Bactrocera correcta*, young sterile, prerelease diet, mating pairs

STARTER DIET AS INITIATING FEED FOR THE *ANASTREPHA LUDENS* LARVAE IN MASS REARING CONDITIONS IN MOSCAFRUT FACILITY.

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Background: The nutritional conditions in insects have a significant role in the ability of males to attract females and result in mating. The relation between nutrition and male reproductive behavior is shown by production of signs for courtship and males may use nutrients to synthesize materials for production of pheromones or substances transferred during copulation such as sperm (Braga, R. 2006). There is a general agreement that protein-fed males are more likely to mate. Vienna-7 sterile Med fly males mate much more frequently in cages with protein than did the regular sugar fed colony. On the other hand wild males that emerged in the laboratory and were tested for mate in a field cage also succeeded significantly better than sugar fed males (Braga, R. 2006). For Moscafrut Facility is important to maintain the characteristics of the flies and improve them at the level with the wild ones, so trying the gelid food as starter diet for the mass rearing to rise *Anastrepha ludens* and *A. obliqua* is expected that it will give the necessary nutriment which the larvae requires, transferring to the final diet (the traditional diet in less proportion) to finish its maturity process; giving the desire mass rearing and quality parameters and in the other hand the high costs of the elaborating diet will be reduced (there is a high consume of imported ingredients for the traditional diet).

Method: After seeding the egg in the jelly diet (Nutryfly®) the new larvae was transferred to the normal diet to finish its development, then it was separated from the diet, continuing with its recovery as pupae and adult. So the parameters that will be tested are: yield egg-larvae, number of larvae per tray and larvae weight. The values of established parameters are: Yield egg-larvae (Y.E-L): 75%; weight larvae: 26 mg; number larvae/tray: 20,900.

Results: From the three egg densities (1.2 ml, 1.4 ml and 1.6 ml) the results were: 1.2 ml: 73.36% Y.E-L, 25.78 mg and 19,273 larvae/tray; 1.4 ml: 66.88 Y.E-L, 25.14 mg and 20,504 larvae/tray; 1.6 ml: 58.86% T.E-L, 24.39 mg and 20,578 larvae/tray. With the data obtained from the 1.2 ml was selected for a second phase to improve it, this because two of the three parameters were close to get, so in the second part the test was divided, a seeding was done in the morning (9:00 am) and the other in the afternoon (6:00 pm) considering the egg hatching; the results were: 1.2 ml in the morning: 69.47% T.E-L, 28.37 mg and 18,211 larvae/tray; 1.2 ml in the afternoon: 75.59% T.E-L, 27.80 mg and 19,958 larvae/tray.

Conclusions: according the results using a low density (1.2ml) it is possible to obtain the production and the quality parameters (even the yield is high and the longevity per cent); if the temperature for the egg hatching (increasing it) can be regulated is possible to improve the results in the starter feed; so it is useful to try 1.2 ml for the further tests between the gelid food as starting feed with the traditional feed and compare the results in a mass rearing system in *A. ludens*, also for *A. obliqua*. Finally other benefits come from this kind of feeding, like the residual reduction of the diet, the minimized import ingredients consumption, the space and the storage are optimized in the areas.

Keywords: starter diet, yield egg-larvae, larvae weight and larvae per tray

EFFECT OF ANOXIA ON MASS REARED QUEENSLAND FRUIT FLY *BACTROCERA TRYONI* (FROGGATT) (DIPTERA: TEPHRITIDAE).

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Background: Area wide treatments using the Sterile Insect Technique (SIT) rely on irradiated or sterile fruit flies to produce no viable offspring in wild fly populations. Irradiation of oxygenated tissues is known to cause adverse effects on fruit fly and need to be minimised. A simplest method to decrease the level of oxygen in pupae is to seal pupae in plastic bags and allow the oxygen levels to decline to the low levels. However some species do not survive well in depleted oxygen for long periods. We report here on tests conducted on *Bactrocera tryoni* (Froggatt) Qfly to evaluate the adverse effects of different periods of anoxia.

Methods: Four experiments were conducted using irradiated and non irradiated Qfly pupae at 17°C, 21°C and 25°C. Anoxia for 56, 96, 168 and 192 hours were tested. At the end of each period of anoxia, the bags were opened and eclosion allowed to occur. After full eclosion, the percentage successful eclosion was assessed and analysed statistically.

Results: In the first experiment using unirradiated pupae for up to 56 hours of anoxia, the highest average eclosion of 89.5% occurred at 21°C. In the second experiment using unirradiated pupae at up to 96 hours of anoxia, there was a decline from 89.4% eclosion at 0 hours to 78.1% at 96 hours with again 21°C resulting in higher eclosion. There were small changes in the percentage of partially eclosed adults but larger increases in uneclosed pupae. In the third experiment, irradiated and unirradiated pupae were compared up to 192 hours of anoxia at only 17°C. The average eclosion declined by 14% to about 70%. Unirradiated pupae had 8% higher average eclosion compared with irradiated pupae. The fourth experiment used only irradiated pupae at 17°C. Full eclosion at 0 hours was 78.0% and declined to 64.6% at 192 hours.

Conclusions: Optimum eclosion appears to occur at 21°C. Eclosion declined at approximately 0.1% eclosion per hour of anoxia during the first 96 hours. There was a 12-14% decline in eclosion after 192 hours of anoxia. These results are at variance with many other species. Qfly are tolerant of long periods of anoxia and current practices will not disadvantage eclosion rates as long as temperatures are kept at optimal.

CAN STERILE SPERM PRESENCE AND FRUIT DAMAGE BE USED AS INDICATORS OF EFFICACY OF MEDFLY SIT PROGRAMS?

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Background: A Sterile Insect Technique (SIT) programme against the Mediterranean fruit fly, *Ceratitis capitata*, has the goal of decreasing the target population by area-wide release of large numbers of sterile males. These sterile males will mate with wild females, which will then produce unviable eggs and consequently a decrease of the wild population. Success of a SIT programme requires a continued evaluation of its efficacy which is currently evaluated by monitoring of both wild and sterile males by means of pheromone baited traps. In this work, the efficacy in suppression of 5 medfly male ratios (wild:sterile) were evaluated in a citrus field experiment. For this aim, the correlation between sterile males mate achievement, measured by molecular sperm identification in trapped wild females, and the production of viable progeny measured as pupae emerged from sentinel fruits, was obtained.

Methods: The experiment was conducted in 17-y-old single mesh-caged clementine trees. In each cage (3 x 3 x 3 m) 50 wild males and 50 wild females with the corresponding sterile males (ratios of 1:0; 1:1; 1:5; 1:10 and 1:20 wild: sterile) were released. Ten susceptible fruits were hold on each tree to count after exposition the number of emerging medfly pupae per fruit. The presence of Vienna-8 sterile male sperm in the spermathecae of collected female was detected by specific PCR markers.

Results: The number of medfly pupae emerged per fruit was significantly different between treatments. Treatments of 1:5 and higher ratios showed a number of pupae emerged significantly lower than treatments 1:1 and 1:0 where no sterile males were released. The percentage of population reduction was around 80% for the 1:5; 1:10 and 1:20 (wild: sterile) treatments when compared to trees with no sterile males releases. Accordingly, percentages females with presence of sterile sperm were detected in those treatments where sterile males were released. Among ratios tested, number of pupae emerged per fruit was related to percentage of sterile sperm detection.

Conclusions: The 1:5, 1:10 and 1:20 (wild: sterile) *C. capitata* males ratios effectively reduced medfly populations and this was reflected in a reduction in fruit damage. The results of the experience showed a relation between fruit damage and sterile sperm molecular identification. Implications of these results for a SIT programme are discussed.

Keywords: *Ceratitis capitata*, sterile insect technique, (wild: sterile) male ratio, medfly infestation, mating success, PCR sperm detection.

ADVANCES IN THE MASS REARING OF *ANASTREPHA SERPENTINA* (WIEDEMANN) (DIPTERA: TEPHRITIDAE) IN PERU.

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Background: Agricultural activity in the Peru has acquired great economic importance since it generates foreign exchange through export; this activity has been limited by the presence of fruit flies of the genus *Anastrepha*. In the Peru, one of the *three* species of major economic and quarantine importance is *Anastrepha serpentina* which causes strong damage to fruit species such as *Lucuma obovata* and *Sapotaceae* other, causing serious economic losses in the Peruvian farmers. Therefore, in the Laboratory of Methods Development SENASA is carry research related to colonization, mass rearing, sterilization behavior and fruit fly *Anastrepha* complex with the objective of implementing the Sterile Insect Technique (SIT) to control this pest. Here is the advance in mass rearing *A. serpentina* achieved in Peru.

Methods: The colony established and maintained in the laboratory was obtained from pupae recovered from infested fruits from the field (of two places: Cajamarca and Ayacucho). Having obtained the adult flies, the first problem was to obtain the eggs of *A. serpentina* form artificially. We tested different panels of oviposition (two colors mesh Bombay). Once approved this panel, were tested for hatching of eggs and artificial diet was developed to carry out the life cycle of flies in the laboratory.

Results: It was possible to establish the environmental conditions for this species, temperatures and humidity of the environments used for each stage of *A. serpentina* ranged from 19-28° C and 60-85% relative humidity. From the 5th generation began to collect eggs in the oviposition panel: Bombay blue mesh recommended by other researchers and as the cages have been used, oviposition increased, reaching up to 1.5 ml collecting egg per day from 4 cages with an average of 32 000 adults in each cage, from infested fruits in the laboratory. The artificial diet tested was composed of: hydrolysate enzymatic protein, sugar, methylparaben, sodium benzoate, citric acid and “chala” (leaves and stems of corn dry and milled) as texturizing agent, we were able obtained pupae with a weight average of 15 mg and a 70% of emergency.

Conclusions: It is possible the progressive adaptation of *A. serpentina* under laboratory conditions for subsequent mass rearing in our country. The selected artificial diet is still in testing because it requires fine-tune the quantities of inputs to use and conduct more tests with other types of inputs to achieve a greater recovery of larvae and adults.

Keywords: *Anastrepha serpentina*, fruit fly, oviposition, Sterile Insect Technique

PRE-RELEASE PROTEIN DIET, ALSO A KISS OF DEATH FOR *ANASTREPHA LUDENS*?

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Background: Protein supplements offered during the pre-release period have been shown to improve male mating competitiveness in *Ceratitis capitata* and *Bactrocera tryoni*. However, protein during pre-release could be a “kiss of death” for flies if they are released into a stressful environment where they may starve if food is scarce. Here, we tested the sexual performance and longevity of sterile *Anastrepha ludens* (aka Mex fly) males and females fed sugar and protein (hydrolysed yeast) during different days during the six-day pre-release period and then starved until sexual maturity.

Methods: Sterile males and females were offered protein and sugar for varying numbers of days during the pre-release period for *A. ludens*. Protein was offered at the beginning of emergence or before release in a total of 10 combinations. After 5 days, all flies were starved and offered water only until 10 days of age. Control flies were fed continuous protein and sugar or sugar only until 10 days of age. Daily survival was noted. The number of copulations of males and females from each treatment were recorded for flies that survived until 10 days of age.

Results: Preliminary results suggest that flies fed on sugar and protein for 5 to 3 days and then offered water only did not survive to sexual maturity. However, males fed sugar and protein for only two days immediately after adult emergence or immediately before release did survive on water only and were more competitive sexually than males fed sugar only. Furthermore, females fed protein during two days did not mate.

Conclusions: Providing protein supplements for a short period of time to females may not be enough for them to mature sexually, which could favor a unisexual release in this species. Protein supplements provided for only two days immediately after emergence or immediately before release may be enough to increase the sexual performance of sterile males without the detrimental effects on longevity.

Keywords: Mexfly, protein diet, sexual competitiveness, longevity

EXPOSURE OF STERILE MALES TO GINGER ROOT OIL REDUCES MATING IN WILD *CERATITIS CAPITATA* FEMALES (DIPTERA: TEPHRITIDAE).

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Background: Females of *Ceratitidis capitata* are facultatively polyandrous, with remating more common in laboratory strains rather than wild ones. In the application of the Sterile Insect Technique (SIT) against this pest, large overflooding ratios of sterile: wild males can increase the remating frequency, because females that mate for the first time with a sterile male tend to remate more frequently. The exposure of sterile males to ginger root oil (GRO) is used in *C. capitata* SIT programmes to increase the sterile male's mating success. Exposing males to an aromatherapy with GRO results in greater competitiveness of sterile males when competing for wild females, and this may also increase the remating frequency among wild females.

Methods: This work examined, under greatly male biased sex ratios, whether sterile male exposure to GRO has an effect on the remating behavior of wild females. The frequency of wild females remating, number of matings per female, the refractory period between the first and second mating, and the duration of the first and second matings of wild females were determined under laboratory conditions for three mating scenarios that included wild males only or wild males competing with sterile males (either GRO-treated or non-treated).

Results: Wild females first mated with sterile males exposed to GRO had their remating rate over six days and the mean number of matings per female reduced in comparison to those first mated with non-exposed sterile males, from 62.5% to 31.1% and from 3.1 to 1.6, respectively.

Conclusion: The remating parameters of females mated with sterile GRO-exposed males resembled those of females mated with wild males.

Keywords: *Ceratitidis capitata*, female receptivity, sterile insect technique, α -copaene

EFFECT OF METHYL EUGENOL EXPOSITION TO YOUNG STERILE MALES OF *BACTROCERA DORSALIS*(HENDEL) AND *BACTROCERA CORRECTA* (BEZZI) ON MATING SUCCESS.

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Background: Area-wide integrated control of fruit flies are going on in Thailand while Area-wide Sterile Insect Technique (SIT) program against fruit flies are increasingly implemented worldwide. Achievement of SIT is to assess mating success of released sterile males. Influence of methyl eugenol, ME, was generally known as effective to the sexual maturity male fruit flies. We reported here on effect of methyl eugenol on young sterile males as a precise marker to assess mating performance.

Methods: Premature mass reared sterile males of the 32nd and 34th generations of two species tephritid fruit fly, (Diptera: Tephritidae), *Bactrocera dorsalis* (Hendel) and *Bactrocera correcta* (Bezzi), respectively were fed only water-agar after eclosion. The first group of sterile males, 2, 3, 4 and 5 day-old, were exposed to 1 ml ME from 8.00-9.00 a.m. The second group of the same ages were not exposed to ME and provided with standard adult diet, sugar protein hydrolysate, SP 3:1, and water *al libitum*. During 15 testing days of 50 *B. dorsalis* sterile males without ME, 50 sterile males with ME, and 50 mature wild males (23 day-old) were introduced into field cage competing for 50 of mature wild females (21 day-old). *Bactrocera correcta* were conducted and observed similar as *B. dorsalis*. But the testing day for *B. correcta* took 18 days; mature wild males were 34 day-old; mature wild females were 37 day-old.

Results: *Bactrocera dorsalis* sterile males, 2 day-old, exposed to ME showed significantly higher percentage of mated pairs than ones achieved by non methyl-eugenol and set of 3+n, 4+n and 5+n day-old males after exposed 2-15 days. Higher percentage of mated pairs were shown on the first day after exposed than the group without ME and reached no significantly different on the 5th-8th and 10th, 13th-15th day after treated. Enhanced mating performance of older fly *B. dorsalis* strongly presented for 15 days. Meanwhile sterile males exposed to ME at age 3, 4 and 5 days were started no significant different from the 3rd, 2nd and 2nd day after treated, respectively. For *B. correcta*, percentage of mated pairs of all young sterile males exposed to ME at age 2, 3, 4 and 5 day-old were significantly higher than the ones achieved by non ME and reached non significant to wild males from the 6th-14th day. Older *B. correcta* flies treated at age 3, 4, 5 day-old showed high peak of the relative sterile index, RSI, that was non significant with wild males from the first day after treated. The relative sterile index of sterile males treated at age 3 and 4 days was better than wild males from the 8th-13th day and the 6th-11th day after treated, respectively.

Conclusions: Young sterile males of *B. dorsalis* and *B. correcta* of 2, 3, 4 and 5 day-old exposed to ME showed manifest mating competitiveness significantly higher than non ME exposed steril males. But they were competitively inferior to mature wild males.

Keywords: *Bactrocera dorsalis*, *Bactrocera correcta*, young sterile male, methyl eugenol expose, mating competitiveness

INFLUENCE OF GINGER ROOT OIL EXPOSURE AND POST-TENERAL NUTRITION ON LONGEVITY AND MORTALITY IN BAIT TREATMENTS OF STERILE MEDFLY.

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Background: A key issue for Sterile Insect Technique (SIT) programmes against medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is to release sterile males that are sufficiently competitive with males from the wild population. Post-teneral nutrition and ginger oil (GRO) exposure of sterile males prior to release have been shown to improve male competitiveness or performance. However, few studies are available on the effect of post-teneral nutrition and ginger oil exposure on longevity and mortality in bait treatments by sterile male *C. capitata*. Our study was based on the hypothesis that the increase of sexual activity of males exposed to GRO may have an energetic cost that induces them to search for food resources, such as proteins included in bait treatments.

Methods: Four treatments were compared: (1) protein-fed flies not exposed to GRO, (2) protein-fed flies exposed to GRO, (3) protein-deprived flies not exposed to GRO, and (4) protein-deprived flies exposed to GRO. Longevity and mortality in bait treatments of sterile medfly of the Vienna 8 strain for the corresponding treatments were obtained.

Results: Sterile male longevity was increased by the addition of protein to the standard pre-release sugar diet, whereas exposure to GRO did not influence their longevity. Mortality in spinosad baits was influenced both by diet and GRO exposure. Sterile males on a protein-deprived diet suffered greater mortality than sterile males fed with both sugar and protein. When sterile males were fed on the protein-deprived diet, GRO exposure increased their mortality. However, no significant differences were found in adults on the sugar-protein diet, whether or not they had been exposed to GRO.

Conclusions: These results show, for the first time, a negative effect of GRO exposure in terms of increasing mortality in proteinaceous bait treatments, a common practice in areas where SIT is implemented. Nevertheless, this effect could be reduced by the addition of protein to the standard pre-release diet. The implications of these results for SIT programmes against *C. capitata* are discussed.

Keywords: Sterile Insect Technique, SIT, medfly, sugar, protein, proteinaceous bait, Diptera, Tephritidae

PROTEIN RESOURCE ALTERNATIVES FOR MASS REARING OF *CERATITIS CAPITATA* WIEDEMANN (DIPTERA: TEPHRITIDAE).

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Background: *Ceratitis capitata* is one of the most economic importance pest in fruit crops around the world and it's control need to be a safety and environmental friendly technique, such as Sterile Insect Technique (SIT), that consists to mass rearing insects with high quality insect and low cost. Artificial diet is the major bottleneck in the Facility Moscamed Brazil (BMB), regarding the source protein (yeast hydrolysate) of the adult's diet, because it is expensive and has to be imported.

Methods: Two commercial products, from national origin and low cost, were tested (yeast autolysed-AL and yeast extract-EL) as substitutes of imported protein. The biological parameters appraised were: fecundity, adults' longevity and eggs' viability.

Results: We founded that flies mass reared on the national protein sources were equivalent or superior to the flies on imported one, where females fed on EL protein in laboratory conditions presented higher fecundity and females fed on EL protein had higher longevity.

Conclusions: Based in the obtained results the two commercial products demonstrated to be possible substitutes for imported protein (yeast hydrolysate) in *C. capitata* mass rearing, which can be easily found in the national market, and with low cost.

Keywords: Mediterranean fruit fly, medfly, adult diet, facility, SIT.

IMPROVED AND DEVELOPED OF ALTERNATIVE LARVAL DIETS FOR MASS REARING FRUIT FLIES SPECIES OF THE GENUS *ANASTREPHA*.

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Background: Mass rearing fruit flies for Sterile Insect Technique programs needs artificial diets to maintain and developed larvae of the target species. The feasibility of any diet depends on the cost of the raw materials, their accessibility and quality. The Moscafrut Facility at Metapa, Chiapas, México has a continuous project for test available and developed new ingredients with potential to use for mass-production *Anastrepha ludens* and *A. obliqua*, and to maintain colonies of *A. serpentina* and *A. striata* with the goal to increase quality and decrease the cost.

Methods: The work consisted of six experiments. In the first was determined the efficacy of new diets, jellified diet using corn, rice and potato starch (Nutrifly[®]), jellified diet using no purified agar (Agarmex) and liquid diet. In the second experiment were evaluated no typical protein sources, Tempeh (flour of chick pea fermented with *Rhizopus oligosporus*), Mubarqui[®] (flour of *Amaranthus* seeds), lyophilized of fruits of *Sargentia greggi* and yeast extract; compared with Torula yeast and hydrolyzed protein. In the third experiment was evaluated the agar-started diet. In the fourth experiment was evaluated the elimination of the Methyl Paraben (Nipagen). In the fifth experiment was evaluated the Torula yeast content. In the last experiment were evaluated new commercial available torula yeast marks.

Results: At experimental conditions the Nutrifly[®], liquid and agar diets increased the larval weight maintained the at least 70% of neonate larval survival for *A. ludens*, *A. obliqua* and *A. serpentina*. The Tempeh permitted high larval transformation and pupal weight in *A. obliqua*. The use of agar-started diet permitted the established of an experimental colony of *A. striata*. The absence of Nipagen had not any effect of the production and quality parameters. *A. ludens*, *A. obliqua* and *A. serpentina* required diets with 9, 7 and 5% content, respectively. Diets prepared with Lake States[®], Nutribio[®] or Lallemand[®] yeast produced excellent results in experimental tests for *A. ludens* and *A. obliqua*.

Conclusions: Jellified diet with starch and agar have high potential to use as started diet, which as liquid diet permit save space and production cost. Tempeh, Mubarqui[®], and lyophilized of fruits need to improve but today they could be used to substitute partially the torula yeast. Started diet permits high larval survival during the colonization and establishment of new colonies. Nipagen is not necessary for larval survival in the diet. Lake States[®], Nutribio[®] (Biosaf) and Lallemand[®] yeast permitted survival larvae. Protein sources as distiller's dried grains and soluble (DDGS) should be tested.

Keywords: *Anastrepha*, larval diets, protein sources, started diets, torula yeast

EFFECT OF ENSILED RICE STRAW AND FRUIT FLY LEFTOVERS ON BEEF CATTLE PERFORMANCE.

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Background: Fruit fly mass rearing need a large amount of larva diet which is wheat bran based diet. Spent larva diet or fruit fly leftovers (FFL) still contain nutritive value for livestock as shown by proximate analysis of 57.98 % moisture, 12.7 % crude protein, 1.86 % fat, 19.36 % fiber, 7.84 % ash, 0.05 % calcium and 0.2 % phosphorous. Application of fruit fly leftovers as livestock feed could save cost of environmental problem in term of disposing the used diet and save feed cost of livestock production.

Methods: Change over design was conducted to evaluate the effectiveness of 4 treatments. A; ensiled 95 kg. of 4 % urea-rice straw + 5 kg. of malasses (control). B; ensiled 60 kg. of 5 % urea-rice straw + 5 kg. of malasses + 35 kg. of FFL. C; ensiled 45 kg. of 5 % urea-rice straw + 5 kg. of malasses + 50 kg. of FFL. D; ensiled 30 kg. of 5 % urea-rice straw + 5 kg. of malasses + 65 kg. of FFL. 16 hybrid cattle were assigned as individual effect and 4 phases of feeding were assigned as row effect. Each phase of feeding was 4 weeks with 2 weeks of resting period.

Results: Four treatments of cattle feed show gradually decreased from 8.5 (A; control), to 7 (B; 35 % FFL), 6.5 (C; 50 % FFL), and 5.5 (D; 65% FFL) corresponding with increasing of strong smell of ammonia. The first 3 groups of ensiled feed were yellow-to-brown in color whereas feed D produced brown-to-dark brown color. Average daily gain (kg./day) of feed A, B, C and D were 0.790, 0.710, 0.840 and 0.620, respectively and found significant difference ($p < 0.05$) between C and D. Feed intake per head per day of A and C were 8.02 and 8.01 kg. which were significantly higher than 6.75 kg. of B ($p < 0.05$). Feed conversion ratio of A, B and C were 10.64, 10.24 and 10.75, respectively, which were significantly lower than 14.13 of D ($p < 0.05$). Cost of 1 kg body weight gain was economically found in C (€0.93) which were lower than € 1.02, €1.1 and €1.14 of A, B and D, respectively.

Conclusions: Fruit fly leftovers contain nutrients which can be made good feed for beef cattle. Ensiled 45 kg. of 5 % urea-rice straw + 5 kg. of malasses + 50 kg. of fruit fly leftovers showed suitable performance in term of growth rate and cost of production.

Keywords: larva diet, wheat bran based diet, fruit fly leftovers, ensiled urea- rice straw, beef cattle

PERFORMANCE OF GROWING SWINE FED BY DIETS SUPPLEMENTED WITH DIFFERENT LEVELS OF FRUIT FLY MASS-REARING DIET LEFTOVERS.

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Background: Larva diet, wheat bran based diet, in process of fruit fly mass rearing consist of wheat bran, granulated sugar, instant dry yeast, sodium benzoate, methyl-p-hydroxybenzoate, acetic acid and water. Nutrients in the used larva diet or fruitfly leftovers(FFL) were shown by proximate analysis of 57.98 % moisture, 12.7 % crude protein, 1.86 % fat, 19.36 % fiber, 7.84 % ash, 0.05 % calcium and 0.2 % phosphorous with amino acids profile of 0.44 % alanine, 0.31 % arginine, 0.13% cystine, 0.37 % glycine, 0.32 % histidine, 0.22 % isoleucine, 0.56 % leucine, 0.23 % lysine, 0.13 % methionine, 0.31% phenylalanine, 0.27 % threonine, 0.13 % tryptophan, 0.18 % tyrosine and 0.31 % valine. The mentioned nutrients are benefit to produce swine feed, especially feed for growing- finishing swines since major cost of production of this period is feed cost.

Methods: Randomized complete block design was applied to evaluate experimental feed. Growing swines, 60 kg. of body weight, were divided in to 4 groups. Each group was composed of 8 swines. Treatments were grower diet varied by different levels of dried fruit fly leftovers as followed : treatment 1, grower diet with 20 % rice bran without FFL (control); treatment 2,

grower diet with 7.5 % rice bran + 7.5 % FFL; treatment 3, grower diet with 0 % rice bran + % 15 FFL; treatment 4, grower diet with 0 % rice bran + 20 % FFL. Each diet group was fortified by 3,200 kg cal/kg ME, 18 % protein and other nutrient requirements followed by NRC(1998). All of swines were fed by full feeding until about 100 kg. of body weight.

Results: Palatability of diets mixed with FFL were a bit better than control group resulting in higher feed intake and faster growth rate in term of average daily gain which were 0.872, 0.967, 0.970 and 0.851 gram/day for control, 7.5 % FFL, 15 % FFL and 20 % FFL, respectively. Growth rate of 7.5 and 15 % FFL were significantly higher than 20 % FFL ($p < 0.05$). Feed conversion ratio of diet with 20 % FFL indicated the poorest efficiency with the number of 3.24 whereas the other groups were very close to 3.02. Feed cost per gain of 15 % FFL got the lowest price which was significantly lower than control group ($p < 0.05$). About carcass quality expressed as back fat thickness were range from 9.5 to 10.0 cm. which were not significantly different ($p > 0.05$).

Conclusions: Nutrients in spent larva diet or fruitfly leftovers was very close to rice bran which can be used as feed stuff in swine feed formulation. Supplementation of 15 % FFL in grower diet resulting in growing swines showed the best performance in term of feed intake, growth rate, feed efficiency and feed cost per gain.

Keywords: Fruit fly leftovers, grower diet, growth rate, feed efficiency, feed cost per gain

SEXUAL MATURATION IN A MUTANT STRAIN OF *ANASTREPHA FRATERCULUS* (DIPTERA: TEPHRITIDAE).

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Background: Sexual maturation is a relatively long process in *Anastrepha* males. This leads to an increase in the costs to apply the sterile insect technique (SIT) because the flies must be kept in laboratory conditions for several days before released. Our objective was to study the duration of the sexual maturation process in a mutant strain in *Anastrepha fraterculus* and to study the heretability of this trait.

Methods: To estimate sexual maturity we observed the sexual behavior of males at different ages, after offering them sexually mature virgin females. The percentage of males that mated at different ages was the variable under study. We analyzed two strains: an eye color mutant (#3210) and a wild-type strain as control (L-TUC). We found that males from strain #3210 had a significantly shorter pre-copulatory period than the males from the other strain. To study the heredability of this character in the mutant strain, we performed crosses between individuals from the #3210 strain and the males and females of wild-type stock.

Results: We observed that the sons of #3210 males matured significantly faster than the descendants of wild-type males, irrespective of the origin of the mother. When the descendants of both sexes were analyzed at the 6th and 13th generation, we found that these results were repeated.

Conclusions: These results encourage the use of this strain of *A. fraterculus* both as model to study the inheritance of genes associated to the sexual maturation, and also as a candidate strain for massive rearing and release with the framework of an SIT program against this fruit fly pest.

Keywords: sexual maturation, *Anastrepha fraterculus* mutant, males pre-copulatory period.

Session 6
Area-Wide & Action Programs

SIT PILOT PROJECT IN CROATIA; CONTROL OF MEDFLY BY SIT IN THE NERETVA VALLEY

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Background: The Neretva river valley is divided with a part in the Republic of Croatia and a part in Bosnia and Herzegovina. It has been noted that highest damage caused by medfly – *Ceratitis capitata* Wied. (Diptera, Tephritidae) is along the border area and can cause up to 30% damage on mandarines. The Croatian Government strongly supports the project since the medfly became a serious pest in the valley. Interest from the Croatian Government to integrate the sterile insect technique (SIT) on an area-wide basis as an alternative for insecticide-based medfly control in consultation with the Joint FAO/IAEA Division determined that Neretva river valley could be a good location to apply this environmentally friendly pest control method as part of an integrated pest management system. The Neretva valley, is the largest citrus production area in Croatia.

The Neretva river valley is a relatively isolated ecosystem (similar to that of the Hex River Valley in South Africa where IAEA had supported activities of medfly control by using of SIT). This provides a good probability of success for a future project since such relative isolation by mountains would limit the movement of wild medfly adults into the valley. A national project involving the Neretva Valley as a pilot project for southern Europe is feasible in view that it represents an ecosystem at the northern edge of medfly distribution.

Methods: An pilot area of 650 ha of mandarine was selected as pilot area where sterile males are released since april 2010. The flies were produced in BioFly Israel, and were shipped directly to Croatian airport Split, twice per week. A total of 70 shipments will be delivered to Croatia during 2010, as a part of FAO/IAEA RER 5014 TCP.

Croatian government build an emergency facility with capacity of 20 mil flies/week in city of Opuzen in Neretva river valley.

Results and Conclusions: The Neretva valley includes over 80% of the Croatian national citrus production, valued at USD 13 million of exported mandarine fruit per year, mostly to EU. The medfly control by integrating SIT technique would economically benefit all local stakeholders including growers and the public at large in view more access to fresh fruits. This program will benefit from the support of the local authorities as well as of the growers and exporters.

Keywords: Neretva river valley, SIT technique, pilot project

THE CUBANS EXPERIENCES ON MONITORING AND MANAGEMENT OF FRUIT FLY *ANASTREPHA* SPP (DIPTERA:TEPHRITIDAE) IN MANGO (*MANGIFERA INDICA* L) AND GUAVA (*PSIDIUM GUAJAVA. L*) CULTURE.

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Background: Agricultural programs for sustained fruit tree production have increased during last years, contributing to availability of products for consumption and marketing. Tephritid fruit flies constitute one of the main pests of tropical and subtropical fruit trees. The presence of fruit fly species of *Anastrepha* genus in fruit tree culture has originated the application of quarantine regulations and restrictions of importing and exporting countries. Of them, due to importance for Cuban fruitculture stand out seven, being of the greater impact: "Caribbean fruit fly", *Anastrepha suspensa* Loew, "West Indian fruit fly" *Anastrepha obliqua* Mcquart, in guava and mango indistinctly.

Methods: Studies on mango and guava cultures were done with the following objectives: taxonomic identification, monitoring systems (traps, attractants and fruit sampling, occurrence of parasites), population behavior, and prospecting and control methods. Entomological inspections were done to detect the presence of fruit fly parasitoids in traps with attractants and collected fruits. The use of chemical, agrotechnical, cultural biological and postharvest control methods were tested in mango and guava cultures.

Results: The obtained result was that *Anastrepha obliqua* and *A. suspensa* were the species of more frequency in the culture. It is proposed as result the use of Torula yeast as attractant alternative with varieties and contributions to monitoring and sampling system, alternative host plant, population behavior, biological control (prospection & application) with the detection by first time in Cuba the larvae parasite *Utetes anastrephae* (Hymenoptera: Braconidae) and the application of the entomopathogenous nematodes *Heterorabditis indica* on larvae and pupae of these species of fruit flies. The agrotechnical, cultural and chemical (insecticide-bait) methods showed good results. The effectivity of treatment postharvest with the use of hot water on the fruits of mango cv Super Haden, presented 4 for the egg stadium and zero for larvae, while with wax cover, a total of 48 and 53 alive larvae were obtained. In the combinations wax cover + hot water, the control of larvae was of 100%. This effectivity of control pre & postharvest are important and insert in the IPM Program.

Conclusions: The use of alternative attractants, the knowledge of the behavior, hosts plants of *Anastrepha spp*, the agrotechnical, chemical cultural, biological control and postharvest assays offer promissory result in this work line of importance for the economic and quarantine point of view. These results constitute the principal basis of IPM on fruit flies of economic importance in Cuba conditions.

Keywords: *Anastrepha*, monitoring, mango guava, pre and postharvest control

ADVANCES IN THE NATIONAL PROGRAMME AGAINST FRUIT FLIES IN MÉXICO.

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Background: In 1992 the Government of Mexico established the National Program against Fruit Flies (NPPF) in order to control populations of *Anastrepha ludens*, *A. obliqua*, *A. striata* and *A. serpentina*, and establish Free Areas of Fruit Flies (FA-FF) and Areas of Low Prevalence of Fruit Flies (ALP-FF), under an Area-Wide Integrated Pest Management (AW-IPM) approach, including the Sterile Insect Technique (SIT) and the Augmentative Biological Control (ABC). In this paper we report the most significant achievements in the period 2001 - 2010.

Methods: We established strategic alliances among the Federal Government, State Governments and fruit producers, to combine efforts and economic resources to implement the Strategic Plan for the control of the pest. We signed an Agreement Cooperation with the Interamerican Institute for Cooperation on Agriculture (IICA), for the production of sterile flies *A. ludens* and *A. obliqua*, and the parasitoid *Diachasmimorpha longicaudata*. With the Institute of Ecology AC, and the International Atomic of Energy Agency (IAEA) were implemented specific projects to perform basic and applied research. Currently, the SIT is applied on 100 000 ha of citrus, mango and guava, for which we operate five centres of Packaging and Release of sterile flies under the system of chilling adult and aerial releases. On a weekly basis, in this facilities are packed an average of 215 million of sterile pupa, using PARC boxes or towers type “Mexico” or “Worly”. After emergence, the adult flies are supplied with juvenile hormone through the food, in order to accelerate sexual maturity.

Results. In 2009 we produce 9.500 million of *A. ludens* sterile, 2.300 million of *A. obliqua* sterile and 1,600 million of *D. longicaudata*. Until May 2010, 49.91% (978.093 km²) of the national territory has been recognized as free area of fruit flies (FAFF), and 10.7% (210.603 Km²) as low prevalence areas of fruit flies (ALP-FF). From free areas, we have been exported 160 000 tons of mango, 120 000 tons of orange, and 7.300 tons of peach, with a trade value of U.S. \$ 190 million. In our area of Methods Development, we have more strains of parasitoids (e.g., *Coptera haywardi* and *Doryctobracon crawfordi*), which in short time could be scaled to mass production with the aim to strengthen the biological control of the pest. In our project of genetic sexing, we obtained a new strain of *A. ludens* which is differentiated by black pupae in females, which allows us the exclusive selection of males. This condition is a relevant attribute for the SIT, so we have started evaluating the quality of the strain, and scaling it for mass production.

Conclusions. The economic evaluation of this Programme in six states of the Mexican Republic during the period of 1994-2008, demonstrates the benefits of the implemented actions, which reflects that investment has a positive cost-benefit ratio.

Keywords: Area-Wide, IPM, SIT, *Anastrepha* spp. Pest Free Areas.

SMALL ACREAGE AREA-WIDE CONTROL OF *BACTROCERA DORSALIS* HENDEL ON CITRUS.

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Background: Citrus is a very important fruit crop in Taiwan with an acreage of more than 32,000 ha. The oriental fruit fly (*Bactrocera dorsalis* Hendel, OFF) is a constant problem on the crop, and since citrus is such an important export commodity, the OFF control program has been on the priority list ever since 1959. Following the success of area-wide control of OFF in sugar apple (in Taitung) and wax apple (in Juan-Nan), similar studies to control OFF damage on citrus were deemed appropriate.

Methods: Da-Lin Township of Chia-Yi County was selected for this study, because citrus is the only fruit crop grown in this area. With the cooperation of area growers, an area-wide OFF control program was launched in 2005. It included a four step process: (1) Growers were organized into a cooperative control team. (2) OFF population densities were monitored, and appropriate geographic and agricultural data were collected. (3) Beginning two months prior to and lasting until the end of harvesting, methyl eugenol bait was used to attract and kill OFF. (4) Field sanitation. The control program was carried out in a 400 ha test area which included 120 ha citrus orchards (60,000 trees) beginning in early June, 2005. No buffer zone was set as protect orchard and spatial warming.

Results: The citrus production in Da-Lin was not subjected to bagging as was done in the wax apple and sugar apple studies. As such, the OFF damage rate was assessed directly. The OFF population was reduced to less than one fly per trap per day in comparison to historical data. By sampling the citrus fruits from three orchards, a fruit damage rate of 0.35% was observed. An additional benefit of this program was that no insecticides were needed. Reducing the OFF damage minimized not only the insecticide cost, but eliminated the insecticide residue problem as well. The quality of the citrus fruits was also improved. For each of the two consecutive years, eliminating the use of insecticide spray resulted in a cost saving estimated at 1.4 million NT dollars (US\$ 44,343)/ year .

Conclusions: Economic motivation and grower education are critical incentives in a successful area-wide control program. The success of such small acreage control programs can be used as a model for extension into other similar production areas in Taiwan in future studies.

Keywords: Area-wide control, *Bactrocera dorsalis*, Citrus.

MANAGEMENT OF FRUIT FLY, *BACTROCERA CUCURBITAE* (COQUILLET) INFESTING GHERKINS USING AREA WIDE CONTROL.

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Background: The melon fly, *Bactrocera cucurbitae*, is a serious pest of gherkin (*Cucumis sativus*), which is cultivated by small individual farmers but collectively on a large scale through contract farming in southern peninsular India for export to world markets. Intensive control measures undertaken by individual gherkin farmers were insufficient as fruit damage was above 4%, and highly unacceptable to the export market. An area wide management (AWM) program using a combination of male annihilation technique (MAT), protein bait application technique (BAT) and sugar baits was thus attempted for more effective management of the melon fly. We report here on the success of this area wide management program in suppressing melon fly populations, and reducing and maintaining melon fly damage on gherkin at levels acceptable for export markets.

Methods: The area wide management (AWM) program was undertaken over an area of approximately three square km in Kashapura village of Gauribidanur taluk, Chickaballapura district, southern Karnataka, over the period April 2008 to March 2010. The AWM area comprised gherkin (10%), other crops (50%) and fallow area (40%) within a central zone surrounded by a 50m buffer zone. The control methods used were male annihilation technique (MAT), protein bait application technique (BAT), and sugar baits. Male annihilation was achieved through cue lure + chlorpyrifos blocks (Nomate life time* from Agri Land) placed at 50 m intervals within the central zone, and 25 m apart within the buffer zone. The cue lure blocks were replaced monthly. Protein bait (Prima protein bait from Malaysia) plus malathion spot sprays were applied at weekly intervals to the gherkin crop. In addition, a sugar (10 % jaggery) plus malathion solution was applied to the maize grown as a border crop surrounding gherkin. The same treatments were imposed in individual gherkin plots situated 3 km away from the AWM area. To evaluate the effectiveness of the AWM program, melon fly populations (number of fruit flies trapped per day - FTD) were monitored continuously using cue lure traps that were cleared weekly. Levels of fruit fly damage to gherkin fruits at harvest were assessed by dissection of fruits in the factory..

Results: Treatments with MAT, BAT and sugar bait sprays in the AWM area trapped 0.9 fruit flies per day during first week of January 2008 and this was reduced to zero during the last week of April 2010. In comparison, treatments with MAT, BAT and sugar bait sprays in the control area outside the AWM area still trapped 0.02 flies in the last week of April 2010. In harvested gherkin fruits, fruit fly infestation in the AWM area was reduced to 0.3 %, which is acceptable by the factory compared to an infestation level of 1.5% in the control area.

Conclusions: Area wide management with treatments, MAT, BAT and sugar bait sprays were highly effective in reducing infestation by melon fly in gherkin to less than 0.3 per cent, and enabled the gherkin industry in southern India to meet with the stringent quality required by international export markets.

Keywords: *Bactrocera cucurbitae*, MAT, BAT, Area wide management, FTD.

ESTABLISHMENT OF A COLONY OF THE NEW WORLD SCREWORM, *COCHLIOMYIA HOMINIVORAX*, FOR COMING AW-IPM PROJECTS IN

MERCOSUL.

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Background: Since the 1950's, the AW-IPM programs integrating the SIT against the New World Screwworm (NWS), *Cochliomyia hominivorax* (Diptera: Calliphoridae), have been successful. The NWS was eradicated from the USA, Central America to Panama, but in most tropical regions of Latin America (excluding Chile), the NWS is still a serious threat to livestock. There is a raising interest for its suppression in South America. Between January and May 2009, a pilot-project, supported by the BID and COMEXA, was performed at the Brazil-Uruguay border. As the results were positive, novel regional AW-IPM projects can be planned. To set a mass-rearing center based in South America is strategic when considering long-term programs. The CENA/USP has a pilot-facility, built by the Agriculture Ministry and supported by FAO/IAEA, which serves to give technical support to SIT projects adopted by the government. In partnership with the Biofactory MOSCAMED Brazil, a project to produce sterile NWS started on 2009.

Methods: The project aims to maintain a colony of a regional NWS strain, to develop a mass-rearing system and a sterilization protocol by X rays, and to study the sterility induction in regional strains. So far, a colony has been successfully established. The parental generation came from UNICAMP pupae (originally from Goiás State, Central Brazil). The adults are kept in cages (90x145x75 cm) and fed on a diet (honey, spray dried blood and brewer's yeast-Bionis[®]). The oviposition substrate is a mix of larval diet disposal and blood. The larvae grow in a medium made of spray dried blood, spray dried egg, milk, sodium citrate, citric acid, water, formalin and ground meat (7%: 2%: 1%: 0.1%: 0.1%: 100%: 0.1%: 70% (w/v)). On the 5th day, larvae pupate in vermiculite. The colony is maintained at controlled rooms (38±1°C; 60±10% RH).

Results: The colony has been reared for 7 generations. Egg hatch has been of 70±10%. From G₁ to G₇, the total amount of pupae produced was about 8 L (~ 67,000 pupae). The mean adult emergence and sex ratio (♀/♂+♀) were 86.7±3% and 0.59±0.08 respectively. Currently, only 1.5 L pupae/generation is produced (cost ~ US\$ 35.00), what is enough to conduct the rearing and sterilization bioassays. Tests to eliminate the meat from the larval diet and to introduce local bulk agents (as sugarcane bagasse and coconut fiber) are underway and will decrease the rearing cost.

Conclusion: This NWS colony will be soon transferred to the BMB to attend coming SIT projects in MERCOSUL.

Keywords: SIT, Screwworm, *Cochliomyia hominivorax*.

AREA-WIDE *BACTROCERA CUCURBITAE* (COQUILLET) CONTROL IN SELECTED AREAS OF MAURITIUS.

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Background: The melon fly, *Bactrocera cucurbitae* (Coquillett), is the most important insect pest of cucurbit crops in Mauritius (Mascarenes island in the Indian Ocean) causing heavy damage. To control this pest, planters have recourse to regular conventional insecticide cover sprays with all their disadvantages. Besides, this practice adds up to their cost of production. A project entitled Feasibility Study for the Suppression of the Melon Fly in Selected Areas of Mauritius – MAR 5/016 was launched in June 2007. The main objectives of the project are to produce quality cucurbits through effective environment-friendly suppression methods and to minimise the use of pesticides for melon fly management.

Methods: An isolated area of 110 ha occupied by some 135 cucurbit growers was selected. The growers were sensitised on the project through meetings, pamphlets, stickers and video film. As an incentive to participate fully in the project, they were all given sprayers, fruit disposal cages, protein bait and MAT blocks. The growers were introduced to the techniques used in area-wide melon fly suppression. Gradually they started to adopt the recommended methods, which included targeted bait applications, male annihilation technique and sanitation. Fruit flies were monitored through dry traps baited with Cuelure and Malathion, wet traps baited with modified waste brewer's yeast and fruit collection and incubation in the laboratory.

Results: Cucurbit infestation which was above 30% before project implementation was reduced to 5% one year later. A decrease in the frequency of pesticide application was noted. 25% of the planters were treating their crops thrice a week before the project was started compared to 5% one year after project implementation. An increase in cucurbit production was noted. 85% of planters claimed to have obtained an increase in both quality and quantity of cucurbits. The melon fly population was reduced by 50%.

Conclusions: Cucurbit growers were able to produce quality cucurbits by adopting environment-friendly techniques of melon fly control. The cost of cucurbit production was reduced through a decrease in use of pesticides as well as application costs. The project has demonstrated the use of an integrated approach to reduce insecticide use, and to produce better quality fruits.

Keywords: Melon fly, trapping, bait application, male annihilation technique

NEW ACHIEVEMENT OF FRUIT FLY SUPPRESSION PROGRAM IN THAILAND: CASE STUDY IN SAMUT SAKORN PROVINCE.

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Background: Fruit fly control has been focused for more than 20 years in Thailand using the Sterile Insect Technique-SIT, along with other safe methods. The successful technologies conducted in pilot areas have been conveyed and modified to be suitable in each other areas. Base on knowledge of area-wide fruit fly integrated control, Samut Sakorn province was proposed, which is traditional scatter fruit orchards over more than 100 km². Various fruits grown in this province are major hosts of tephritid fruit fly (Diptera: Tephritidae). Fruit production in this area is presented all year round causing of fruit fly outbreak and fruit damage which might be very high up to 100 percent if there is no any insecticide applied. Sprays and young fruit bagging are normally used but costly to control fruit fly infestation. Samut Sakorn Provincial Administration Organization has embarked and supported the growers for controlling Tephritid fruit fly by collaboration with the Department of Agricultural Extension, Ministry of Agriculture and Cooperatives.

Methods: To decrease fruit fly population, the committee of Samut Sakorn Provincial Administration Organization suggested that various aspects, outbreak problems by growers, growers's attitude on fruit fly control methods and etc., should be considered and then growers would be educated on cause of the outbreak, suitable and effective fruit fly control and growers participation. To increase empowerment of growers, at the beginning of activities, growers were participated activities together, taught to make traps using polyethelene terephthalate-PET with methyl eugenol as a fly attractant. Growers were motivated by given fertilizer, value 1,000 Baht/ 2 litre of flies, as award if they could show captured fruit flies from their own handmade traps.

Results: Growers in Samut Sakorn province were interested in all activities organized by the committee. They gained the knowledge, continue to cooperate and also help each other in controlling fruit flies. However, main achievements are the changed of growers' attitude on mass trapping using methyl eugenol, decreasing of fruit fly population, lower fruit infestation and better visible quality.

Conclusions: Campaign of area-wide fruit fly mass trapping could be simply implemented by growers and local government cooperation. Convincing the growers to use fruit fly traps and put more effort following neighbour success would be the way to stimulate them. Samut Sakorn Provincial Administration Organization has made a plan to establish fruit fly surveillance using trapping network and implementation of other appropriate environment friendly technologies.

Keywords: fruit fly suppression, Samutsakorn province, mass trapping, area-wide, methyl eugenol.

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EVALUATION OF ENTOMOPATHOGENIC FUNGI TO OF *CERATITIS CAPITATA*.

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Background: Laboratory experiments were carried out to measure the pathogenicity and to study the infection process of isolate 412 of *Beauveria bassiana*, and isolate V24 of *Lecanicillium muscarium* to old larvae of the Mediterranean fruit fly, *Ceratitidis capitata* (Diptera: Tephritidae) under the laboratory conditions after inoculation of the fungal suspension on the soil surface.

Methods: To evaluate the potential of the fungi to old larvae, plastic doses (3.8 cm × 2.8 cm) were filled with dry soil and sprayed with 3 ml suspension (1×10^8 Spores/ml) on the soil surface using a small dash bottle. The density of spores valued (2.6×10^7 Spores/cm²) on the soil. After soil application the old larvae were put on the treated soil. The plastic doses were then incubated at 25°C in the dark. Nine days after the treatment, the number of pupae, moulded and emerged flies were detected. Dead pupae were cut and the condition determined. The flies were transferred in cages and provided with tap water and a diet of yeast.

Results: In these trials *L. muscarium* and *B. bassiana* were pathogenic to *C. capitata* after the contact of old larvae on the soil. Non dead or moulded Larvae were found in the varieties. All of the contaminated larvae could develop to pupae, but the death occurred by pupae stage. The mortality of pupae ranged between 51.6 % by *L. muscarium* and 46.7 % by *B. bassiana* in comparison to the control with 18.4 %. The development of infected flies inside the pupae was stopped 2 to 3 days after the pupation. Among of dead pupae 35 % by *L. muscarium* and 26.6 % by *B. bassiana* were moulded. The fungal growth of *L. muscarium* and *B. bassiana* was clearly inside the pupae. In some cases the mycelium emerged from the pupae. This fact indicates that the spores were picked up from the soil and adhered on the body of larvae, but the infection process occurred 2 to 3 days after the pupation inside the pupae, where the body of fly was formed.

Conclusions: The results showed that a soil application of *L. muscarium* and *B. bassiana* could reduce the emergency of fruit fly of *C. capitata*. About 48.3 % of pupae were emerged by *L. muscarium* and 53 % by *B. bassiana*, while 82 % of pupae were emerged in the control. The following experiments should be carried out to determine the effect on the eggs, pupae and the flies.

Keywords: entomopathogenic fungi, Mediterranean fruit fly, mortality, Mouldiness

EFFECT OF SOIL APPLICATION OF *PAECILOMYCES LILACINUS* (THOM) SAMSON IN CONTROLLING MELON FLY *BACTROCERA CUCURBITAE* COQ.

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Background: Melon fly *Bactrocera cucurbitae* (Diptera: Tephritidae) is a serious pest of cucurbitaceous vegetables causing 30 to 100 per cent crop loss (Sookar *et al.*, 2006). Annual loss in fruit and vegetable production equivalent to Rs. 26,902 million has been reported during the year 2000 in India (Stonehouse, 2001). Although management options like hydrolyzed protein spray, parapheromone trap (Zaman 1995; Stonehouse *et al.*, 2005), field sanitation (Akhtaruzzaman *et al.*, 2000), chemical spray (Waseem *et al.*, 2009) have been adopted, it is ideal to develop an eco-friendly and user-friendly method for the management of fruit flies. Various Deuteromycetes fungi viz., *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces fumosoroseus* were known to cause epizootics in fruit flies (Dimbi *et al.*, 2003; Yee and Lacey, 2005). Jiji *et al.*, (2006) reported the pathogenicity of *B. bassiana*, *P. lilacinus*, *Aspergillus candidus*: Fries on melon fly. The study was undertaken to explore the efficacy of *P. lilacinus* in the management of melon fly.

Methods: The bio-efficacy of the entomopathogenic fungus *P. lilacinus* was tested on the pupae of melon flies under *in vitro* conditions. Glass troughs (30 cm diameter) were filled with soil to a depth of 4 cm. The soil was drenched with 30 ml of spore suspension at 1.3×10^7 , 1.3×10^8 , 1.3×10^9 spores/ml. Soil drenched with sterile water served as control. Ten numbers of one day old pupae were introduced into the soils on the third, fifth and seventh day after treatment of the fungus. Observations on the number of infected pupae per trough were recorded on the tenth day. Based on *in vitro* studies, the fungus was tested under field conditions. Bran based formulation of the fungus was applied as soil drenching (1.3×10^9 spores / ml) at fortnightly intervals during the fruiting period of bittergourd. Observations on the number of infected pupae / sq.m was recorded.

Results: When the pupae was introduced on the third day after inoculation of the fungus significant mortality (92.45% at 1.3×10^9 followed by 72.28% at 1.3×10^8) was observed which was on par (69.84% at 1.3×10^7 spores/ml) with that on the tenth day after treatment. When pupae were introduced on the fifth day after inoculation of the soil with the fungus significant mortality of 69.84% at 1.3×10^7 , 74.92% at 1.3×10^8 and 92.45% at 1.3×10^9 was recorded on the tenth day. When pupae were introduced on the seventh day after inoculation of the fungus, mortality percentage of 32.30, 44.62 and 67.28 at 1.3×10^7 , 1.3×10^8 , 1.3×10^9 spores/ml, respectively was recorded on the tenth day. Under *in vivo* conditions the number of infected pupae varied from 2.30 to 3.99 per sq.m.

Conclusions: Soil drenching with *P. lilacinus* is effective, eco-friendly and easily amenable method that fits well into the IPM schedule for the management of fruit flies. The same fungus was found pathogenic to *B. dorsalis*, the findings of which widens the scope of its practical utility.

Keywords: *Bactrocera cucurbitae*, *Paecilomyces lilacinus*, melon fly, bittergourd, cucurbits

PERFORMANCE OF TWO INTRODUCED PARASITIODS AGAINST *BACTROCERA INVADENS* ON DIFFERENT HOST FRUITS AND TEMPERATURES.

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Background: The horticulture industry across sub-Saharan Africa is plagued with fruit fly pests, and the situation has worsened in recent years with the arrival of the invasive *Bactrocera invadens*. The failure of native parasitoids to effectively control this alien species led to the introduction of two co-evolved parasitoids (the egg-prepupal parasitoid *Fopius arisanus* and larval-prepupal parasitoid *Diachasmimorpha longicaudata*) for evaluation against *B. invadens*. Laboratory tests showed that these parasitoids especially *F. arisanus* are promising candidates for management of *B. invadens*. However their performance on various host fruits and temperature remains to be elucidated. This information will help in guiding the releases of this parasitoid as part of IPM management strategy for *B. invadens*.

Methods: Performance of the two parasitoids as measured by percent parasitism, total number of parasitoid progeny, sex ratio, and developmental time were assessed across four host fruits: mango, pawpaw and citrus. The suitable stage of *B. invadens* was exposed on the fruit peels placed on an oviposition unit to female wasps in no choice experiment. After 24 hours, the exposed hosts were incubated till parasitoid and fruit fly emergence. Total number of emerged parasitoids (male and female), their developmental time, and unclosed puparia were recorded. Percent parasitism and sex ratio were computed for the two parasitoids on the four host fruits. The methodology was the same for the temperature experiments except that parasitized *B. invadens* eggs or larvae were incubated at varying temperatures.

Results: Results showed that *F. arisanus* parasitizes eggs of *B. invadens* reared on mango, pawpaw and citrus resulting in a mean % parasitism of 63.8%, 62.4% and 24.0% respectively. The % parasitism for eggs reared on citrus was significantly lower compared to mango and pawpaw and this may have been due to the significantly low number of pupae recovered in citrus experiments. Parasitism rates of 34.9%, 25.4%, 23.2% and 18.4% for mango, pawpaw, guava and citrus were recorded with respect to *D. longicaudata* with the rate for mango being significantly higher than the other fruits. For temperature experiments, 35°C was found to be detrimental to the development of parasitized *B. invadens* eggs and larvae, as all eggs and larvae were unable to complete the full developmental cycle. Temperature of 25°C was the most favourable for the development and rearing of both *F. arisanus* and *D. longicaudata*.

Conclusions: *F. arisanus* and *D. longicaudata* are effective biological control agents against *B. invadens*. With the exception of citrus which is not favourable for the development of *B. invadens*, the two parasitoids were effective against *B. invadens* eggs reared on mango and pawpaw fruits. The two parasitoids were relatively tolerant to temperature conditions that exist on the African continent.

Keywords: *Bactrocera invadens*, *Fopius arisanus*, *Diachasmimorpha longicaudata*, host fruits, temperature

RADIATION DOSES TO SAFELY RELEASE THE PARASITOID *DIACHASMIMORPHA LONGICAUDATA* REARED ON *ANASTREPHA FRATERCULUS* LARVAE.

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Background: *Anastrepha fraterculus* is a fruit fly of economic importance in Argentina and other countries in South America. Biological control programs based on the release of the braconid *Diachasmimorpha longicaudata* have been successful to control other flies of the genus *Anastrepha*. In order to release this parasitoid it is necessary to establish the radiation dose that, both, avoid fruit fly emergence from non parasitized pupae and, do not affect parasitoid performance.

Methods: *A. fraterculus* larvae were irradiated with X-ray, at doses equivalent to: 60, 80 and 100 Gy (6250.2, 8333.6 and 10417.0 roentgens, respectively). Immediately after irradiation, larvae were offered to *D. longicaudata* mated females. A control group of irradiated larvae were not exposed to parasitoids. Number of emerged parasitoids, flies and non emerged pupae were registered. Emerged flies (if any) were crossed with non-irradiated flies and egg production and egg hatch were registered. For those parasitoids that emerged from irradiated larvae fecundity was determined. Fecundity was assessed by offering host larvae every other day, during 2 weeks.

Results: Larvae irradiated at 60 Gy showed high level of emergence and were as fertile as control (non-irradiated larvae). At 80 Gy, only 1.4% of the pupae emerged, males having a significantly reduction in fertility. No flies emerged from larvae irradiated at 100 Gy. Parasitism rate on larvae irradiated at 80 or 100 Gy did not differ from that found in control (non-irradiated) larvae, nor did their offspring's sex ratio. F1 fertility was also not affected by the radiation dose.

Conclusions: These results suggest that irradiation with X-rays at a dose equivalent to 100 Gy applied to third instar larvae of *A. fraterculus* can significantly reduce the risk of release of adult fruit flies on the field without affecting the development and subsequent performance of *D. longicaudata*. This dose could be reduced to 80 Gy, as emergence will also be close to zero and emerged flies will be to a large extent sterile.

Keywords: *Anastrepha fraterculus*, *Diachasmimorpha longicaudata*, Biological Control, X-ray

STUDY ON THE ACTIVITY OF THE PUPAL PARASITOID *SPALANGIA CAMERONI* (HYMENOPTERA: PTEROMALIDAE) AS A POTENTIAL BIOLOGICAL CONTROL AGENT OF THE MEDITERRANEAN FRUIT FLY *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE).

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Background: In spite of the lasting presence of the Mediterranean fruit fly, *Ceratitis capitata*, in the Mediterranean area there were not references for native parasitoids of the pest in Spain. The pteromalid *Spalangia cameroni*, has recently been identified (in 2003) as a parasitoid of *C. capitata* in Spain. This insect is well known as a pupal parasitoid of flies belonging to different taxonomic families: Muscidae, Sarcophagidae and Tephritidae. But it was not known its parasitism on *C. capitata* until now. Due to the importance of the Medfly as a serious pest on citrus and fruit trees in the Community of Valencia (Spanish eastern coast), the parasitic activity of this parasitoid on *C. capitata* is being studied in laboratory conditions for the last 5 years to assess its potential as a biological control agent of the Mediterranean fruit fly.

Methods: In this work we present data about several parasitic features (fecundity, fertility, host-age and host number incidence, ...) in the parasitism of *Spalangia cameroni* on pupae of *Ceratitis capitata* in controlled conditions (in the laboratory), as well as on the use of frozen pupae of the Medfly to be applied to the mass rearing of the parasitoid.

Results: In general, results show a good activity of the parasitoid on *C. capitata* in the laboratory. Also, the use of frozen pupae reveals as a good method to facilitate the mass rearing of the parasitoid.

Conclusions: We can conclude that this parasitoid could be an interesting candidate to be used in the biological control of the Medfly in Spain, but some work in field conditions must be done to assure its real effectiveness against the pest.

Keywords: *Ceratitis capitata*, *Spalangia cameroni*, parasitic activity, mass rearing.

DIPLOID MALES IN *DIACHASMIMORPHA LONGICAUDATA* (ASHMEAD) (HYMENOPTERA: BRACONIDAE).

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Background: *Diachasmimorpha longicaudata* is an endoparasitoid of last instar larva of tephritid fruit flies. It is widely used as biological control agent of these pests, and has been massively produced in several bio-factories. One of the facts that should be taken into account in order to optimize its production is the possibility of generating a bias on the sex ratio in favor of females, as they are the ones that will attack and cause the death of the host. In this direction, it is necessary to study the sex determination system in the species. Hymenopterans are characterized by being haplodiploid (females are diploid and come from fertilized eggs, and males are haploid and come from unfertilized eggs). Nevertheless, diploid males have been observed in more than 70 species. According to one of the hypotheses about sex determination in Hymenoptera, sex is determined by the status of the sex determination locus/loci. When heterozygous, the individual will develop as female, when hemizygous, as male, and when homozygous, as diploid male. Considering this, the production of diploid males should be induced by an increase in the level of endogamy. These males are considered as a genetic load for the population as they are usually infertile. They also cause enormous losses in massive production, as they come from fertilized eggs that could have become females. The ploidy level of an individual can be established by analyzing its chromosome number. According to previous cytogenetic studies performed on *D. longicaudata*, males have $n=20$ chromosomes and females have $2n=40$ chromosomes.

Methods: In order to determine whether diploid males are produced in *D. longicaudata*, 12 crosses between virgin females and one of their haploid sons were performed. Their male descendants were studied by cytogenetics, and their ploidy level was recorded. Chromosome preparations were made by spreading of testis from prepupa, pupa and pre-emerged adults, dyed with DAPI and photographed.

Results: 10 out of the 12 crosses produced male and female descendants, indicating that the female was fertilized. Per couple, between 3 and 21 individuals were karyotyped. A total of 108 chromosome preparations were performed, and from them, 49 corresponded to haploid males, 6 to diploid males ($2n=40$ chromosomes), 9 were recorded as haploid males but with not enough certainty, and other 9 were identified as diploid males in the same conditions. The rest of the individuals (35) didn't show cells in metaphase stage, so it wasn't possible to determine their ploidy level.

Conclusions: These results constitute the first description of diploid males in *D. longicaudata*, and set a starting point to study the sex determination system in this species in order to optimize the rearing protocols applied up to now.

Keywords: Biological control, sex determination, diploid male

INCREASE OF PARASITISM RATES OF *DIACHASMIMORPHA LONGICAUDATA* REARING ON X-IRRADIATED *CERATITIS CAPITATA* LARVAE.

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Background: Control strategies including the sterile insect technique (SIT) and biological control using parasitoids are currently applied against *Ceratitidis capitata*. In Argentina, the larval parasitoid *Diachasmimorpha longicaudata* is currently being evaluated as candidate for biological control. Parasitism rate is one of the most important parameter to determine productivity and efficacy of parasitoids as biocontrol agents. The study of host-parasitoid interactions provides useful information to optimize the massive rearing of parasitoids. This interaction involves the immune response which is triggered after the female parasitoid oviposits on the host larvae. Previous studies have shown that the exposure to radiation suppresses host immune system responses in other insect species. Our objective was to study parasitism rates by *D. longicaudata* on irradiated larvae of *C. capitata*.

Methods: Late second stage-early third stage of *C. capitata* larvae were treated with different X-ray doses (20, 40 and 80 Gy) prior to exposure to *D. longicaudata* females. Seven replicates were tested for each dose. Parasitized larvae were kept at controlled condition of humidity and temperature until emergence. Non-irradiated larvae were used as a control. Parasitism rates were calculated from the number of emerged parasitoids for each X-ray dose. The parasitism rate was corrected taking into account the number of non-emerged parasitoids. A one-way ANOVA was performed. When the test was significant, *post-hoc* pair-wise comparisons were performed using Tukey's HSD (Honestly Significant Difference).

Results: Parasitism rates of larvae exposed to 20 G and 40 Gy and non-irradiated larvae showed no significant differences. Larvae irradiated with 80 Gy showed the highest rate of parasitism which significantly differed from those obtained with non-irradiated larvae ($p < 0,01$) and larvae irradiated at 20 Gy and 40 Gy ($p < 0,01$). When compared the corrected parasitism rate, larvae exposed to 20 G and 40 Gy and non-irradiated larvae showed no significant differences. Larvae irradiated with 80 Gy showed the highest rate of parasitism which significantly differed from those obtained with non-irradiated larvae ($p < 0,01$) and larvae irradiated at 20 Gy and 40 Gy ($p < 0,01$).

Conclusions: X-radiation at a dose of 80 Gy affected the parasitism rate of *D. longicaudata* on *C. capitata*. Irradiation effects could be related to suppression of immune response in treated larvae. This result will allow improvements on massive rearing of *D. longicaudata* using *C. capitata* as its host. We will continue this study in order to go deeper in immune response mechanism in *C. capitata*.

Keywords: *Ceratitidis capitata*. *Diachasmimorpha longicaudata*. Immune response. X-radiation.

INCIDENCE OF PARASITOIDS OF *ANASTREPHA* SPP., IN BACKYARD ORCHARDS IN THE SOUTHEAST OF CAMPECHE, MEXICO.

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Background: The fruit flies of the genus *Anastrepha* spp. are the principal phytosanitary problem in fruit crops in tropical and subtropical areas in Latin America, this insects attack a wide range of exotic and native host plants for oviposition. The geographical position of the Mexican state of Campeche in the tropics, with its proximity to the Gulf of Mexico and the Caribbean Sea, determine the conditions for the proliferation of many species of the genus *Anastrepha* spp. This aspect gives us the opportunity to study commercial and native host plants for fruit flies detection in backyard orchards.

Methods: Fruit collections were made in backyard orchards located in the southeastern state of Campeche, Mexico (18 ° 29 '251 " N and 90 ° 55' 309 W, 42.6 meters above sea level, with warm sub-humid climate and summer rains). The collection period was from June 2008 to July 2009. Fruits were collected from *Pouteria sapota*, *Manguifera indica*, *Prunus domestica*, *Citrus paradisi*, *Citrus aurantium*, *Psidium guajava*, *Talisia olivaeformis* and *Zuelenia guidonia* trees. The material collected was transported to the laboratory of the Escuela Superior de Ciencias Agropecuarias, Universidad Autónoma de Campeche. The fruits were dissected and inspected for fruit flies larva presence. Third instar larvae were located in plastic container (5 x 5 cm) with a mesh cover for fruit flies and parasitoids emergence.

Results: All species of commercial and native fruits sampled were attacked by *Anastrepha* fruit flies. The species reared were *A. ludens*, *A. serpentina*, *A. obliqua*, *A. striata* and *A. fraterculus*. The larval parasitoids *Doryctobracton aerolatus*, *Aganaspis pelleranoi* and *Odontosema alvinerve* were reared.

Conclusions: In the Mexican backyard orchards in Campeche, *A. fraterculus* can complete its biological cycle in many commercial and native fruit hosts. The parasitoids *Doryctobracton aerolatus*, *Aganaspis pelleranoi* and *Odontosema alvinerve* were associated to *A. fraterculus*.

Keywords: *Anastrepha* hosts, larval parasitoids

TOXICITY OF SPINOSAD ON *SCAPTOTRIGONA MEXICANA*, MELIPONID BEES (HYMENOPTERA: APIDAE).

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Background: Spinosad (GF-120; Dow AgroSciences,* NF Naturalyte 0.24 g ai/L) is used to control outbreaks of *Ceratitis capitata* in the border Mexico-Guatemala, as part of the Medfly program activities against this pest. Although this product is considered to be selective and harmless to European bees, little is known about the impact on populations of other associated insects in fruit orchards, such as meliponid bees *Scaptotrigona mexicana* (Hymenoptera: Apidae), a common pollinator agent in rambutan and mango orchards in Chiapas, Mexico.

Methods: This work was carried out in the gardens of Moscafrut SAGARPA-IICA Program located in Metapa de Dominguez, Chiapas, Mexico. Meliponid bee hive was provided by the laboratory of apidology of El Colegio de la Frontera Sur. Bioassays were carried out using cylindrical acrylic cage 9 cm in diameter and 8 cm high. We evaluated treatments for fresh and 48 h after application of spinosad, at concentrations of 10, 20, 40, 80 ppm ai. The exposure of the product within each cage (20 mL) was carried out in a plastic slotted cylindrical piece of 1.2 cm in diameter and 10 cm long. For each bioassay, ten repetitions of each concentration were performed, recording mortality at 24 and 48 h.

Results: Mortality of adult meliponid exposed to spinosad residues in fresh showed no difference between the different concentrations and control. When the exposure time was increased up to 48 h no differences among treatments were obtained. Similarly, exposure of dry residues did not significantly affect the survival of insects. When the recommended dose to control fruit flies (80 ppm) was tested, mortality of meliponid bees exposed to either dry or fresh residues was not higher than in control.

Conclusions: Survival of adult *Scaptotrigona mexicana* exposed to residues of spinosad, was not affected significantly when the product was applied according to manufacturer's recommendations.

Keywords: Pollinators, stingless bees, *Scaptotrigona*, toxicity, Spinosad

HORIZONTAL TRANSMISSION OF FUNGAL INFECTION BETWEEN *CERATITIS CAPITATA* ADULTS UNDER LABORATORY CONDITIONS.

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Background: The Mediterranean fruit fly (medfly), *Ceratitidis capitata*, is widely distributed around the World and is regarded as the most important pest of agricultural resources. Its management is still internationally based on treatments with broad-spectrum chemical insecticides. Even though the contact pesticides are commonly effective at higher doses, they cause environmental, ecological and human health problems. Therefore, alternatives strategies have to be developed urgently to control this devastating fruit pest. Biological control by using entomopathogens could represent a suitable alternative strategy to chemical control of fruit flies. Until now, no efficient method was used for the application of entomopathogenic fungi in the field. Dissemination of conidia from infected male or female to its uninfected opposite sex among medfly population could be a potential method. Our investigation aims (i) to evaluate the possibility of horizontal transmission between medfly adults, (ii) to examine whether the medfly male or female could be an efficient spreader of conidia and (iii) to determine how horizontal transmission was affected by the infected males/females ratios and the inoculation method.

Methods: Fresh conidial suspensions of Moroccan *Beauveria bassiana* isolates were prepared and their germination percentages were estimated. Emerged medfly adults were inoculated with 2×10^6 conidia and then coupled with clean opposite sex. Infected male insects were mated with clean females at different ratio: (i) 5 males:5 females, (ii) 5 males with 10 females, (iii) 5 males with 25 females and (iv) 5 males with 50 females. Medfly Infection (mycosis) and Mortality were evaluated using wet and dry conidia.

Results: Horizontal transmission of *Beauveria bassiana* isolates, between different sexes of *C. capitata*, was evaluated in the laboratory. Uninfected males and females could become infected when mated with infected opposite sex. The infection percentage of uninfected males and females ranged from 55% to 76.67% and from 58.33 to 75.00%, respectively, whereas the average of survival time (AST) varied between 11.80 to 15.10 and 13.50 to 15.34 days, respectively. Although, the males or females transmitted equally the infection to their opposite sex during mating, the infection percentage of females was found closely correlated to the ratios of inoculated males to uninfected females. Furthermore, the horizontal transmission of conidia was significantly different between *B. bassiana* isolates. Nevertheless, the mycosis percentage increased when dry conidia was used (66.67-100%) compared to wet conidia (26.67-93.33%). Moreover, the ASTs were shorter with dry conidia (2.36-8.62 days) compared to wet conidia method (12.37-20.06 days).

Conclusion: Horizontal transmission was proved among medfly adults at the laboratory and varied significantly in relation to the *B. bassiana* isolates. The infection percentage and AST of uninfected females were correlated positively to the ratios of infected males. This work revealed that horizontal transmission could be a useful method to management of *C. capitata* in field.

Keywords: *Beauveria bassiana*, *C. capitata*, horizontal transmission, biological control, Argan forests.

CONSERVATION OF PREDACIOUS SPIDERS FOR CONTROLLING FRUIT FLIES IN MANGO ORCHARDS.

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Background: Fruit flies (Diptera: Tephritidae) are the major pests of various fruits in Thailand. Two species of tephritid flies, the Oriental fruit fly, *Bactrocera dorsalis* and the guava fruit fly, *Bactrocera correcta* have been found in mango orchards all year round. The dominant species is *B. dorsalis*. Female fruit flies cause damage by laying their eggs in ripe or nearly ripe fruit. The larval stage lasts about one week then leaves the fruit and drops into the ground to pupate in the soil. Many species of spider role as a predacious spider in mango orchards.

Methods: To study on the outbreak and population density of fruit flies in mango orchard, steiner bait traps with methyl eugenol and insecticide were placed throughout the year in mango orchard areas in Chachoengsao province. The spiders were collected by beating the brunches of mango trees and by sweeping on the weedy areas at unsprayed and spayed orchards. The species and number of fruit flies and predacious spider were surveyed and collected.

Results: the Oriental fruit fly, *B. dorsalis* and the guava fruit fly, *B. correcta* were the most important fruit flies pests in mango orchard. *Bactrocera correcta* was found at the average of 9.68 - 44.73 flies/trap/day during March - May 2008, the seasonal harvesting period of mango in Thailand. Sixty six species of 50 genus and 17 families of spider fauna were found inhabiting in mango orchards. The lynx spider, *Oxyopes lineatipes* is the most important predacious spider in consuming fruit flies. The immature stage, adult females and males of the lynx spider consume fruit flies 7.78, 7.67 and 6.53 flies/day, respectively. In the orchard, spiders stay on weed waiting for newly emerged fruit flies climbing up from the ground. In the unsprayed orchard, high density of spider was found on weed under the tree canopy as well as at the water ways' side areas, whereas many spiders were found on weed at water ways' side areas in the sprayed orchard. In order to conserve the predacious spiders in mango orchards, we concluded that weeds should be kept at certain spots as to provide a shelter for them.

Conclusion: *Bactrocera dorsalis* is the dominant fruit fly species and the lynx spider, *O. lineatipes* is the important natural enemy in mango orchard. The immature stage, adult females and males could consume fruit flies 7.78, 7.67 and 6.53 flies/day. To conserve the predacious spider in mango orchards, weed should be maintained at certain spots as to provide a shelter for them.

Keywords: conservation, *Bactrocera dorsalis* (Hendel), mango

ANALYSIS OF TWO LARVAL-PUPAL PARASITIDS (HYMENOPTERA, BRACONIDAE) IN THE BIOLOGICAL CONTROL OF *CERATITIS CAPITATA* (WIEDEMANN) IN SPANISH MEDITERRANEAN AREAS.

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Background: The Mediterranean fruit fly, *Ceratitidis capitata* (Diptera: Tephritidae), is considered a key pest in fresh fruit and citrus production in the Mediterranean Basin. Nowadays, it is being studied the use of several ecological methods against this pest in some Mediterranean countries (Spain, Morocco and Tunisia), like the Sterile Insect Technique (SIT), the classical biological control (CBC) and the search for native parasitoids. Two exotic larval-pupal parasitoids of fruit-flies have been imported by the IVIA to Spain: *Diachasmimorpha tryoni* and *D. longicaudata*. As it occurs in other countries, in Spain it is being studied the combined use of the SIT and overflooding releases of parasitoids to combat Medfly populations. To achieve this, only one parasitoid species is usually mass reared and released. Over the last two years we have been analyzing the competitiveness of *D. longicaudata* and *D. tryoni* in order to choose the species to be used together the SIT for the integrated control of the Medfly.

Methods: Four factors have been studied in laboratory, greenhouse and field trials to select one species for mass-rearing. These factors are: i) Adult longevity at the four seasons in field; ii) Fecundity and fertility of females in the laboratory; iii) parasitism at variable host densities in laboratory; and iv) interspecific competition in laboratory and greenhouse.

Results: When separately studied in the laboratory both species seems to have similar potential as parasitoids of the Medfly. But in the intraspecific competition assay *D. longicaudata* shows a significant more effectiveness than *D. tryoni* in offspring production (either in laboratory or in greenhouse experiments). This result could mean a competitive superiority of *D. longicaudata*. Field trials have showed an unexpected high longevity in both species but more important in *D. longicaudata*. The experimental releases were performed with a low number of females, but even in this conditions *D. longicaudata* performed better than *D. tryoni*, as only individuals of the previous species have been recovered.

Conclusions: *D. longicaudata* seems to present a competitive superiority in medfly parasitism over *D. tryoni* in Mediterranean conditions. A joint view of all results leads us to select *D. longicaudata* for the application of CBC in the Comunidad Valenciana and open the possibility to use it in the other countries from the north of Africa.

Keywords: *Ceratitidis capitata*, Biological control, parasitoid effectiveness.

INSECTICIDAL ACTIVITY OF TUNISIAN STRAINS OF *BACILLUS THURENGIENSIS* AGAINST LARVAE AND ADULTS OF THE MEDITERRANEAN FRUIT FLY *CERATITIS CAPITATA* WIEDEMAN (DIPTERA: TEPHRITIDAE).

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Background: The Mediterranean fruit fly, *Ceratitis capitata* is one of the key pests on citrus and several others fruit in Tunisia. Chemical control using a broad-spectrum of insecticides remains the most used tool for the management of this pest. However, problems related to the appearance of insect resistance, emergence of secondary pests, impact on non target organisms, environmental pollution and residues further restrict the use of such method. In the aim of searching effective and environment friendly alternatives, the bacterium *Bacillus thuringiensis* has been successfully employed to control insect pests in the orders of Lepidoptera, coleopteran and diptera including *C. capitata*.

Methods: The insecticidal activity of 8 Tunisian strains of *Bacillus thuringiensis* isolated from various regions of Tunisia and selected among 120 strains was assessed against the third instar larvae and newly emerged adults of the Mediterranean fruit fly *C. capitata*. The strains isolates were tested for their activities by using respectively spore-crystal in suspension and lyophilized. Two methods were used. The first method consists in pulverizing the culture medium by 4 ml of the suspension spore-crystal. The second consists in the mixture of the larvae medium by the lyophilised suspension spore-crystal with increasing concentrations: 0.032; 0.048 and 0.064 mg/g of culture medium. The death rate is noted every 24 h during 3 days.

Results: Results showed that the suspension spores-crystal of *Bt18*, *Bt4* and *Bt21* strains were very effective against larvae with respectively death rates of 85, 90 and 95%. The strains *Bt13* and *Bt3* present a significant death rate with respectively 65 and 70%. Newly emerged adults were less sensitive to all tested strains compared to larvae. The highest toxicity was obtained with *Bt21* with 55% of death. According to the second method, highest toxicities for larvae were also obtained for *Bt18*, *Bt4* and *Bt21* strains with respectively death rate of 80, 85 and 90%. For new emerged adults, highest toxicity was observed with *Bt4* with 45% death rate.

Conclusions: Therefore, the use of *B. thuringiensis* could be integrated with some of these existing methods in an IPM approach to control *C. capitata*.

Keywords: *Bacillus thuringiensis*, biopesticide, *Ceratitis capitata*

FUNCTIONAL RESPONSE AND PATCH TIME ALLOCATION OF THE FRUIT FLY PARASITOID *FOPIUS ARISANUS* (SONAN) ON *BACTROCERA INVADENS* DREW, TSURUTA & WHITE.

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Background: To predict and understand the success or failure of a biocontrol agent it is necessary to study their behavior and mechanisms of decision making. In this study we investigated the functional response of the egg-prepupal parasitoid, *Fopius arisanus*, on *Bactrocera invadens* and the effect of host encounters on the patch residence time. Depending on the distribution of hosts in the field, successful host encounters can have either an incremental or decremental effect on patch residence time. *Fopius arisanus* is known to discriminate between suitable and unsuitable hosts. Hence, encounters with parasitized or unhealthy hosts should lead to a decrease in the patch residence time.

Methods: Seven-day old, experienced females of *F. arisanus* were individually exposed to artificially infested mango domes (3, 6, 9, 18, 27, 54 or 81 eggs of *B. invadens*). The behavior of the parasitoids was recorded until the time the female left the mango dome using The Observer® 2.0 software. To estimate the parasitization rate, host eggs were dissected and the number of parasitized eggs recorded. Since the host density was gradually depleted over time the data were fitted to Royama's equation. The effect of oviposition on the patch leaving tendency was tested using the Cox proportional hazards model.

Results: The efficiency of *F. arisanus* increased from 5.5 to 14.9 parasitized hosts per hour at a host density of 3 and 81, respectively. The mean residence time increased from 1782s for the lowest to 7069s for the highest host density. Results also showed that oviposition events decreased the patch leaving tendency ($\exp(\beta) = 0.67$, $P < 0.0001$) hence increased the responsiveness of the parasitoid towards the patch. According to Royama's model the handling time was 198.8s while from our observations one oviposition took an average of 80.5s.

Conclusions: Our results suggest that *F. arisanus* should be able to contribute to the management of *B. invadens*. The practical application of this finding with regard to field suppression of the pest will be presented.

Keywords: *Bactrocera invadens*, *Fopius arisanus*, functional response, patch time allocation

EFFECT OF SOIL PHYSICAL FACTORS ON THE FATE AND VIRULENCE OF THE MITOSPORIC ASCOMYCETE *METARHIZIUM ANISOPLIAE* AGAINST PUPARIA OF *CERATITIS CAPITATA*.

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Background: As an alternative to chemical control or as part of IPM programs, there is a resurgence of interest in the use of microbial insecticides for biological control of tephritid flies, particularly entomopathogenic fungi (EF). The strategic options in using entomopathogenic fungi in medfly control are aerial applications and autodissemination targeting adults and soil inoculation targeting pupariating larvae and puparia. With this aim, in a previous study, we have found *Metarhizium anisopliae* Sorok EAMa 01/58-Su isolate to be highly virulent against both *C. capitata* adults and puparia. However, factual information on the fate of the conidia after they reach the soil will provide a better understanding of the potential of this *M. anisopliae* strain for being applied as an effective biological insecticide against medfly puparia. In this work, we report on the effect of soil pH and texture on the fate in the soil and virulence of EAMa 01/58-Su strain against medfly puparia.

Methods: Sixteen representative Mediterranean soils were used for the studies the effect of soil type on the adsorption of conidia on the soil. The time course evolution of the number of available conidia in each soil suspension was obtained either under standard saline conditions or flocculation conditions. Then, the movement of conidia in the soil was studied using soil columns with four soils representing sandy acid and alkaline and clay acid and alkaline conditions respectively. These four soils were also used for evaluating the physical conditions of the soil on the virulence of EAMa 01/58-Su strains against medfly puparia in transparent containers (8 by 8 by 5.5 cm), each holding 30 g of soil.

Results: Our results indicate that increasing clay contents in the soil resulted in lower number of available conidia in the soil solution. Even if in the soil columns most of the conidia were retained in the *first* soil layer for all soils, this was particularly significant in the sandy ones. Interestingly, the soil salinity clearly influenced the adsorption on the conidia in the soil. Besides, virulence of this strain against medfly puparia in the soil was also depended on the type of soil.

Conclusions: Our results show the potential of soil treatments with *M. anisopliae* EAMa 01/58-Su strain for the microbial control of medfly puparia even if the soil physical conditions have to be considered to decide on the number of application etc. This strain could be used therefore in a biocontrol strategy targeting both adults in aerial sprays and to the soil beneath the tree for controlling puparia, the soil-inhabiting stage.

Keywords: Medfly, *Metarhizium*, *Beauveria*, soil application, geodesinfestation, puparia

THE STERILE INSECT TECHNIQUE AND PARASITIDS (HYMENOPTERA: BRACONIDAE) FOR THE CONTROL OF THE QUEENSLAND FRUIT FLY, *BACTROCERA TRYONI* (FROGGATT) (DIPTERA: TEPHRITIDAE).

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Background: The Queensland fruit fly, *Bactrocera tryoni* is the most significant pest of edible fruit and fruiting vegetables in Australia. With a decreasing reliance on insecticides and an increase in organic produce, biologically safe options are the way forward.

Methods: The sterile insect technique (SIT) is an environmentally friendly option to control or suppress fruit fly populations or outbreaks and has been used in pest free areas (PFAs) and surrounding buffer zones to target outbreaks and suppress populations respectively. However, international studies have shown that the augmentive release of opiine braconids can suppress fruit fly populations to a greater extent than either technique alone. Parasitoids and sterile insects have the advantage to go into areas where other techniques cannot be applied.

Results: This paper reports on the progress of SIT and parasitoids for the control of *B. tryoni* in PFAs and bordering zones. Methods for rearing and releasing sterile *B. tryoni* have been improved and include studies to improve the performance of the fly. Alternative release methods are also being developed and include chilled adult release. Two species of parasitoids, *Diachasmimorpha kraussii* and *D. tryoni*, not previously recorded in inland New South Wales have been identified, although in numbers insufficient to suppress *B. tryoni* populations to below economically acceptable thresholds. However, these results suggest that these two species are candidates for augmentive release in the region. Trials looking at the biology and ecology of these parasitoids in relation to their potential as biological control agents for *B. tryoni* are ongoing.

Conclusions: Overall, results will be presented in relation to the potential for incorporation of the combined use of SIT and parasitoids into pest fruit fly management programs.

Keywords: opiine, natural enemy, pest free area

SPECIES-SPECIFICITY OF A PCR-BASED ASSAY FOR IDENTIFICATION OF *CERATITIS CAPITATA* (WIEDEMANN) PARASITOIDS.

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Background: The Mediterranean fruit fly (medfly) *Ceratitidis capitata* (Wiedemann) is a key pest of citrus and many other fruits. Two exotic braconid medfly parasitoids, *Diachasmimorpha tryoni* (Cameron) and *D. longicaudata* (Ashmead) were imported into Spain, and are being considered for its implementation jointly with the ongoing medfly sterile insect technique program in Valencia. Currently the identification of parasitoids and estimation of parasitism rates rely on tedious and time-consuming dissection and rearing methods. As a result, there has been increasing interest in the use of molecular methods to identify parasitoids and to assess parasitism rates. The present study describes species-specific PCR primers, based on the ribosomal internal transcribed spacer 1 region sequence (ITS-1), for three species of medfly parasitoids and their use in a multiplex PCR assay.

Methods: Complete ITS sequence has been obtained for several species of parasitoids by amplification with ITS universal primers. Species-specific primers for these species and for *C. capitata* were designed on ITS-1 to use in combination with a reverse universal primer. Multiplex PCR conditions have been set up with parasitoid DNA mixtures. Laboratory experiments have been set up to test the multiplex PCR protocol for each species individually and in competition. Parasitism rates were estimated by both molecular and rearing and dissection methods under the conditions tested.

Results: The primers set developed amplify exclusively sized species-specific PCR products in a single reaction. Sensibility of *D. tryoni* and *D. longicaudata* primers have been established at 1 ng of parasitoid DNA (mixed DNA samples). In laboratory experiments, *D. tryoni* and *D. longicaudata* DNA were detected in *C. capitata* pupae after 48h of parasitism.

Conclusions: The multiplex system developed allows establishing the parasitoid species and the parasitism rate, in a feasible fast manner.

Keywords: Medfly, parasitoids, ITS-1

ASSESSMENT OF THE POTENTIAL NON TARGET EFFECTS OF *METARHIZIUM ANISOPLIAE* (SOROKIN) ON THE BRACONID PARASITOIDS *DIACHASMIMORPHA TRYONI* (CAMERON) AND *D. LONGICAUDATA* (ASHMEAD).

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Background: The demand of insecticide free fresh fruit is encouraging the use of ecologically friendly alternatives against the Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann), such as the sterile insect technique (SIT) and biological control programs. Consequently, two imported braconid species *Diachasmimorpha tryoni* (Cameron) and *D. longicaudata* (Ashmead) started to be released in Valencia in autumn 2008. In addition, the utilization of the entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin is being considered jointly with the ongoing SIT program to control medfly in the Comunidad Valenciana. Accordingly, the potential non-target effects of this pathogen on the released parasitoids need to be evaluated.

Methods: Laboratory assays were performed to assess the pathogenicity of *M. anisopliae* to both parasitoids by exposing them to mycosed cadaver flies. The longevity of both exposed and non-exposed parasitoids was determined. Moreover, the effect of *M. anisopliae* on fecundity, sex ratio and emergence rate of parasitoids was calculated. Multiplex-PCR on the non-emerged pupae of *C. capitata* was used to assess the actual rate of parasitism.

Results: The exposition of the two parasitoids to mycosed flies resulted in *D. tryoni* and *D. longicaudata* differential pathogenicity. For *D. tryoni*, the parasitism rate was not affected regardless of the amount of conidia received. For *D. longicaudata*, fecundity, and parasitism rates were affected when higher conidia contact occurred. In any case, the sex ratio of both parasitoids was affected by *M. anisopliae* exposure.

Conclusions: This laboratory evaluation reveals that both parasitoid species are susceptible to *M. anisopliae*, though further field-cage and open field studies are required to confirm these results.

Keywords: Medfly, parasitoids, entomopathogenic fungus, SIT

MEDFLY IMPACT IN SOUTHWESTERN MOROCCO AND BIOLOGICAL CONTROL WITH HALOPHYTES SPECIES.

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Background: The medfly attack reduces growth capacity of the crops and therefore lower commercial yield. This effect is most important when the attacked species is considered such as high importance economic species, such as Argan in southwestern Morocco. The Argan tree (*Argania spinosa*) is endemic to semi-desert Souss valley of southwestern Morocco. In this area, it plays major ecologic and socio-economic roles. The argan oil is considered as a high-value product for that it's crucial to women's co-operatives in Southwest of Morocco. This economic contribution depends of argan tree productivity which faces to abiotic and biotic factors, especially the dipterans fly: *Ceratits capitata*. The argan tree is considered as the largest fruit fly host knows in the world.

Methods: In order to control this insect we propose a biological one with three halophytes species belonging to two different families. The plant extracts were sequentially partitioned for subsequent bioassay. The active fractions were divided into subfractions using a silica gel column (reverse phase). Toxicity and effectiveness of the subfractions were reported as LC50 and LC90, which represent the concentrations in µg/ml with 50% and 90% medly mortality in 24h, respectively. Both larvae and adult medfly mortality were considered.

Results: We noted that a great insecticidal activity for Tamaricacea species comparing with the studied Chenopodiacea species. The *Tamarix* species showed a strongest larvicidal activity against the second and third instar larvae of *Ceratitis capitata*, two times greater comparing with the other extracts. These same findings were also registered for the adulticidal activity of *Tamarix* species it was 21.3% greater comparing with the tested Chepodiacea species.

Conclusion: These results suggest that tested *Tamarix* species may be considered as an interesting bioinsecticidal product. Application of these products could be very useful to reduce the larvae and adult of *Ceratitis capitata*. Future investigations should aim at testing these products against other fruit fly species and try to resolve the mode of action of these bioinsecticidal compounds.

Keywords: biological control, halophytes, *Ceratitis capitata*, *Argania spinosa*

LONGEVITY OF THREE SPECIES OF TEPHRITID (DIPTERA) FRUIT FLY PARASITOIDS (HYMENOPTERA: BRACONIDAE: OPIINAE) PRESENTED WITH TWO FRUIT-BASED DIETS.

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Background: Opiinae braconids are important natural enemies of *Anastrepha* spp. and mass-reared in several locations for area-wide control. Adult parasitic Hymenoptera often feed on floral and extrafloral nectars and honeydews, but an Old World opiine introduced to the Americas, *Diachasmimorpha longicaudata*, survives on orange juice (*Citrus aurantium*) as it seeps from injured fruit. The New World species, *Doryctobracon areolatus* and *Utetes anastrephae*, are also prominent *Anastrepha* parasitoids. The orientation to and longevity upon diets based on two different fruits were compared and contrasted in all three parasitoids.

Methods: Pulp and juice were provided from a domesticated Old World fruit (*C. aurantium*) that offered little opportunity for shared evolutionary histories, except perhaps with *D. longicaudata*. All three parasitoids were also provided with a less-domesticated New World fruit (guava, *Psidium guajava*) which shared an evolutionary history with *D. areolatus* and *U. anastrephae*.

Results: Both sexes of *D. longicaudata*, from USA and Mexican-derived cultures died when provided *P. guajava* pulp or juice at a rate similar to a water-only control. In the USA, *D. areolatus* and *U. anastrephae*, presumably adapted to the nutrient/chemical constituents of *P. guajava*, also died at a rate similar to that of the water-control. Survival of all three species on *C. aurantium* pulp and juice was greater than on water and longevity often equaled that obtained on a honey+water solution provided as a positive control. The differences in survival on the two fruits suggested that *P. guajava* was either: 1) innutritious, 2) toxic or 3) unattractive. Fructose found in *P. guajava* sustained both male and female *D. longicaudata*. Starved female *D. longicaudata* and *D. areolatus* could be found at *P. guajava* feeding-stations, but when given a choice between fruit both sexes of all species were generally found at *C. aurantium* stations. Thus *P. guajava* contained nutrient sugar and could be located over a distance of at least 15 cm by females of two species. Longevity differences between insects fed on *P. guajava* and *C. aurantium* were most likely due to an undiscovered toxin.

Conclusions: Given the differences in fruit-food quality, adult opiine food sources would not be obtainable at all oviposition sites and more additional foraging for food than previously postulated may be required. Volatiles present in *C. aurantium*, but not *P. guajava*, may be candidate attractants.

Keywords: *Diachasmimorpha*, *Doryctobracon*, *Utetes*, *Psidium*, *Citrus*

DISPERSAL OF STERILE MALES OF *ANASTREPHA LUDENS* (DIPTERA: TEPHRITIDAE) TREATED WITH *BEAUVERIA BASSIANA*.

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Background: Laboratory and field cage studies have shown that sterile males can be used for the horizontal transmission of entomopathogenic fungi, as an additional mortality factor for the control of fruit fly pests. Our goal in this study was to investigate the survival and dispersal of sterile *Anastrepha ludens* males treated with *Beauveria bassiana* conidia under natural field conditions.

Methods: Two central point release-recapture experiments were carried out. In the first, marked sterile *A. ludens* adults treated with three different concentrations of dry *B. bassiana* conidia and untreated sterile flies were released in a central point in a mango orchard and subsequently recaptured in a trapping net around the release point using Multilure traps. In the second experiment, only one conidia concentration was used to treat sterile flies. Field survival and dispersal was estimated from the number of trapped flies.

Results: In the first experiment, the recapture of males treated with low and intermediate concentrations was similar to the recapture of untreated males. The highest conidia concentration resulted in the lowest recapture. However, the dispersal pattern was similar for all treatments. In the second experiment, the recapture of sterile untreated males was significantly greater than the recapture of treated males. The longest distance displaced was 203 and 218 m for treated and untreated males, respectively; but the difference were not significant. Dispersal pattern of both, treated and untreated males was to the south-southeast area. The survival to both, treated and untreated males was similar. Only in one, out of five replicates, the difference was significant.

Conclusions: Horizontal transmission of entomopathogenic fungi by sterile males is feasible. Using low conidia concentrations, the field survival and dispersal of treated males was similar to that of untreated males. These and previous results on mating competitiveness suggest that the performance of conidia treated sterile males is not affected. This new approach could result in a more effective use of the Sterile Insect Technique for the control of fruit fly pests, over all when only males are reared in the genetic sexed strain. Methods need to be developed for conidia application just before release of sterile flies.

Keywords: *Beauveria bassiana*, horizontal transmission, microbial control.

GF-120[®] (SPINTOR-CEBO[®]), AN INTERESTING ALTERNATIVE IN SUSTAINABLE AGRICULTURE FOR THE CONTROL OF *CERATITIS CAPITATA* & *BACTROCERA OLEAE*. SIDE-EFFECTS ON BENEFICIALS.

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Background: Spinosad is a microbial pesticide isolated by fermentation from a soil actinomycete, with a new mode of action on the nervous system (prevention of closing of the nicotinic acetylcholine receptors), high efficacy against tephritid flies and good environmental performance, so it has been authorized in EU Organic Agriculture in 2008. Spintor-Cebo[®], incorporates the active ingredient in low concentration (0.024%) plus a bait (mixture of several components) and it has got definitive registration from the Spanish Ministry of Environment, Rural and Marine affairs since 2009, being currently used for the control of two economically important fruit flies, the Med fly in citrus orchards and the olive fruit fly. The insecticide is applied as ultra low volume bait spraying (water amount under 10 l/ha) to the top of the canopy of trees aiming at controlling adults, and the percentage of the treated surface in aerial applications varies from 25-40% depending on the crop, while in ground applications may cover among 5-10% of the vegetation

Methods: Based on the available information in literature, a summary of its characteristics, performance on fruit flies as well as its side-effects on different developmental stages of the beneficial organisms compared with that of classical pesticides is presented here. Results are mainly based in its residual contact activity on laboratory, semi-field or field and on its persistence, but some information on its toxicity by other exposures routes is presented as well.

Results: Spinosad is a bit more slow acting than former active ingredients used for the control of these two fruit flies (organophosphates and pyrethroids), but its efficacy after 72 h application is comparable and the pre-harvest intervals are very short: 1 day in citrus orchards and 7 days in olive groves, because it degrades quickly. The insecticide is compatible with many enemies, mainly predators belonging to different orders and families and pollinators (honeybee), and it possess a risk to many parasitoids, but age residues are usually not harmful because photolysis is the main way of degradation.

Conclusions: Based on its good environmental performance and efficacy against tephritids, as well as its compatibility with non target arthropods and pollinators, it is a good candidate to be used in the control of *Ceratitidis capitata* in citrus orchards and for the control of the *Bactrocera oleae* in olives, in substitution of conventional products especially in Organic Agriculture where only natural insecticides are permitted.

Keywords: Medfly, olive fruit fly, spinosad bait, side-effects, parasitoids, predators

Session 8

**Chemical Ecology, Attractants &
Other Control Methods**

EFFECTIVENESS OF THE MASS TRAPPING TECHNIQUE IN COMBINATION WITH A NEW INSECTICIDE IN *CERATITIS CAPITATA* MANAGEMENT.

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Background: Mass trapping has been widely used in many Mediterranean areas to control the Mediterranean fruit fly (Medfly) *Ceratitidis capitata* giving satisfactory results when used in combination with the insecticide dichlorvos (DDVP). Due to the new directive 91/414/CEE the utilization of DDVP inside the traps has been banned except in exceptional authorizations. Therefore a new effective insecticide is needed in order to assure the success of mass trapping in this fly species. Here we report the efficacy of another insecticide, deltamethrin, in the Medfly mass trapping.

Methods: Four trials have been performed in different fruit varieties and localities. The assays consisted in the deployment of a certain number of traps baited with a suitable attractant and the insecticide replacing the DDVP applied in the upper part of the trap. All traps were placed in external branches in the southern face of the trees following a homogeneous distribution in the correspondent test plot. Every week a fixed number of traps were checked where the total number of adults captured inside were evaluated differentiating between sexes in most cases. In addition, a fruit damage assessment was performed.

Results: The trials carried out supposed both low and high Medfly population densities. In general, females were captured in a higher proportion than males (65-75%) except at the end of the assays where the percentage of males slightly increased. Furthermore, in those tests where the number of live flies captured in the traps was evaluated a very low proportion of them were observed (approximately 2%), showing this the effectiveness of the deltamethrin. The fruit damage rate in all experiences was rather acceptable, especially in those plots where a high number of traps were positioned.

Conclusions: The efficacy of mass trapping using deltamethrin as a DDVP substitute has been proved in all tests executed. This system has a notable capability for capturing adults in both low and high Medfly populations emphasizing an appropriate female percentage caught in the traps, a general low number of live adults in the devices and a very satisfactory fruit damage percentage. Therefore this mass trapping system comprises a good alternative to the traditional DDVP use and thoroughly compatible with integrated pest management.

Keywords: Medfly, mass trapping, DDVP, deltamethrin

VOLATILE EMISSIONS OF *CERATITIS CAPITATA* (WIEDEMANN): INFLUENCE OF FLY SEX, AGE AND MATING STATUS.

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Background: The courtship behavior and pheromone communication between males and females *Ceratitidis capitata* have been studied for many researches, however, the sex pheromone composition is still a controversial subject. The use of semiochemicals is being extended in the control of Mediterranean fruit fly, so the research of new products affecting the medfly behavior or the composition of the sex pheromone would be interesting to improve these control methods.

Methods: The experimental design to study volatile compounds emitted by *C. capitata* consisted of three factors at two levels: sex (male or female), mating status (virgin or mated) and age (3 or 9 days old). Volatile was collected by solid phase microextraction and the compounds were isolated and identified by gas chromatography coupled with mass spectrometry. The data were analyzed by Principal Component Analysis in order to obtain clusters of compounds with a similar emission pattern. A 3-way ANOVA was applied in order to study in detail the emission pattern of these representative compounds according to sex, age and mating status.

Results: Seventy compounds emitted by *C. capitata* were detected. All compounds were detected in males whereas only 37 were found in female volatile profile. The most abundant compounds detected in the male volatile profile were 2-hexenoic acid, geranyl acetate, ethyl (3E)-3-octenoate and (E,E)- α -farnesene. In female volatile profile, 2-ethylhexanoic acid, trimethylamine, 2,5-dimethylpyrazine and 2-hexenoic acid were the most abundant released compounds.

Conclusions: The compounds were grouped in 16 clusters, determining the emission pattern for everyone. The knowledge of the emission pattern for each compound and which of them have similar profile would be interesting to design the biological assays and the combination of compounds to determine the effect on the Mediterranean fruit fly behavior.

Keywords: *Ceratitidis capitata*, volatile profile, solid phase microextraction, pheromone.

FACTORS WHICH INFLUENCE THE EFFICACY OF MASS-TRAPPING TO CONTROL THE MEDFLY *CERATITIS CAPITATA* (WIEDEMANN).

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Background: We analyzed the spatial distribution of adult *Ceratitidis capitata* (Diptera: Tephritidae) captured in the traps, in Citrus orchards where mass trapping was applied to control the pest, to understand how the method works, to determine the factors which influence it and to suggest changes which could improve its efficacy.

Methods: In Ibiza Island, from 2006 to 2008, we established a mass trapping system of control in 31 citrus orchards. The traps, of the tephri-trap type and baited with Tripack as attractant, were uniformly distributed at a density of 50 per hectare following the standard recommendations. In the orchards, all traps of the mass trapping system were sampled at intervals of 30 to 45 days, by counting all flies captured on each trap. In all, 110 samplings, with 102 traps observed per sampling on average, were carried out.

Results: The establishment of the network of traps causes usually a barrier effect, with reduction in medfly captures in a gradual and rather constant rate from the periphery to the interior of the orchards. The amount of reduction observed is usually low, since the average captures were 3.5 flies per trap and day (ftd) on the external perimetral layer of traps and 2.3 ftd on the second layer of traps, reaching 0.7 ftd on the sixth layer located 70 meters inside the orchard. The following factors were analyzed for the barrier effect on medfly populations: size and shape of the orchard, presence of mature fruits, species and/or variety of fruit, month of the year, time elapsed since the mass trapping was installed, and overall medfly abundance in the periphery. Size and shape of the orchard, and month of the year, showed significant influence: Larger plots and plots with minimum ratio perimeter/area showed higher capacity in reduction of captures, and the efficiency of the system of control increases in June and July.

Conclusions: According to these results, the success of mass trapping can be measured by the capacity to get high levels of reduction between the external and the internal layer of traps in the plot. The mass trapping technique must be applied in large areas, of several tens of hectares, in surfaces with a compact shape in order to minimize the perimeter, and increasing the density of traps in the periphery of the protected area. The traps should be installed when fruit ripening approaches but not before, as no improvement was observed when the period of establishment of the mass trapping in the orchards increases.

Keywords: *Ceratitidis capitata*, spatial distribution, mass-trapping, Citrus, traps

DIFFERENCES IN LAMBDA-CYHALOTHRIN SUSCEPTIBILITY AMONG FIELD AND LABORATORY POPULATIONS OF *CERATITIS CAPITATA* (WIEDEMANN).

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Background: The Mediterranean fruit fly (Medfly), *Ceratitidis capitata* (Wiedemann), is one of the most economically important pests of citrus orchards in Spain. The intensity of insecticide treatments with malathion-lure against *C. capitata* has resulted in the development of resistant populations in some areas of Spain. The situation changed in 2009, since the decision of non-inclusion of malathion in Annex 1 of the Directive 91/414/EEC has resulted in the withdrawal of authorisations for plant protection products containing this insecticide. Several insecticides are approved for Medfly control in citrus crops, but spinosad and lambda-cyhalothrin are the most widely applied alternatives. It is therefore necessary to evaluate the susceptibility to lambda-cyhalothrin of field and laboratory populations in order to determine the effectiveness of this insecticide for Medfly control.

Methods: *C. capitata* infested fruits were collected in commercial fields from different geographical areas in Spain during 2009. The susceptibility to lambda-cyhalothrin (10% w/v, Karate Zeon, Syngenta Agro S.A., Madrid, Spain) of emerging adults from each field was determined by dose-response feeding bioassays and compared to the susceptibility of a malathion-resistant and a susceptible laboratory strain. In addition, the malathion-resistant strain is being maintained under lambda-cyhalothrin selection pressure in the laboratory in order to determine the potential for cross-resistance development.

Results: Populations from Granada, Malaga, Valencia and Lerida showed a LC₅₀ for lambda-cyhalothrin of 144, 202, 134 and 287 ppm, respectively. The susceptibility of these populations is remarkably lower than that of a susceptible laboratory strain (LC₅₀ = 18 ppm). In addition, laboratory selection with lambda-cyhalothrin initiated on a malathion-resistant laboratory strain has decreased its susceptibility to lambda-cyhalothrin from LC₅₀= 98 ppm to LC₅₀=759 ppm, whilst malathion susceptibility was maintained. Interestingly, the esterase inhibitor DEF partially suppressed the tolerance to lambda-cyhalotrin of the selected strain.

Conclusions: The implications for Medfly control of the differences in susceptibility to lambda-cyhalothrin of field populations from different origins, when compared to laboratory strains, are discussed. Our results suggest that resistance to malathion may confer cross-resistance to lambda-cyhalothrin.

Keywords: Medfly, lambda-cyhalothrin, cross-resistance, malathion.

SPINOSAD EFFICACY IN LABORATORY AND FIELD CONDITIONS AGAINST THE MEDITERRANEAN FRUIT FLY *CERATITIS CAPITATA* WIED. (DIPTERA: TEPHRITIDAE).

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Background: In Tunisia, the Mediterranean fruit fly *Ceratitis capitata*, is a serious pest causing quantitative and qualitative losses to several crops, mainly citrus. The chemical control using broad-spectrum insecticides, essentially organophosphates, plays a major role in controlling this pest. However, insect resistance was recorded and control failures have been reported in field situations last years. Among the more used insecticides, malathion [diethyl (dimethoxythiophosphorylthio) succinate] has been declared an ozone-depleting substance and therefore, is being phased out. This work aims to evaluate Spinosad efficacy as an alternative to conventional chemical control in Tunisia.

Methods: The assessment of Spinosad efficacy (dose=10ml/hl) was based on determination of adult and larval mortalities in laboratory together with population reduction expressed by weekly male captures and fruit damage at harvest in the field. Non treated insects were used as control in the laboratory experiment whereas in field conditions trials, a control plot of 0.5 ha area was employed.

Results: Laboratory experiment showed that *C. capitata* larvae were more tolerant than adults. At 72h after treatment, Spinosad achieved 22% of mortality of larvae against 3% for the control, whereas, it leads to 100% mortality for adults against 4% for the control. For the field trial, results indicated significant differences in male captures and fruit damage percentages between Spinosad and the control. Concerning adult males captures reductions of 55.15% were recorded. For fruit damage assessment at harvest, results showed significant differences between the treated and the control fields. The percentage of punctured fruit was 13.13% in Spinosad trial against 20.40% for the control.

Conclusions: Spinosad reduced significantly the damage produced by medfly females, and can be an efficient alternative to chemical control in Tunisia.

Keywords: *Ceratitis capitata*, Spinosad, tolerance, fruit damage

A COMPARISON OF SEVERAL TRAPS AND ATTRACTANTS AS A FIRST APPROACH TO THE USE OF MASS TRAPPING AGAINST THE OLIVE FRUIT FLY (DIPTERA, TEPHRITIDAE) IN THE VALENCIAN COMMUNITY.

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Background: The Valencian Community (on the eastern coast of Spain) is the fourth Spanish region in olive production (for oil and olives). One of the main problems for this crop is the olive fruit fly, *Bactrocera oleae* (Gmelin) and this pest has traditionally been combated in the region by the use of chemicals. Nowadays, it is been studied the technical and economical feasibility in using some ecological methods against the pest. One of these methods could be the mass trapping, so some trials have been started to know its effectiveness.

Methods: The experiment was carried out in two olive plots located in Enguera (Valencia, Spain), an inner town about 70 km far from the Mediterranean coast. Two different olive varieties were in each plot: Arbequina and Villalonga. Five traps (glass MacPahil, Tephritrap, ecological Tephritrap, Easy trap and Olike) and three attractants (Nulure+Borax, Tephri Lure and Ammonium Phosphate), with a total of 15 combinations (trap+attractant) were analyzed. Trials started on 5th June and finished on 31st December. Traps were installed on trees in both plots over an area of about 2.300 m² and every other tree. They were moved between trees through the experiment to allow all trap combinations were in all positions in the plot. Traps were moved, attractants renewed and insects inside traps counted every two weeks.

Results: Data were similar in the two plots, so in the two olive varieties. The best trap and the best attractant in capturing olive flies were Tephritrap and Tephri Lure respectively. In addition, the best combination was Tephritrap + Tephri Lure. Ammonium Phosphate shows the worst result in capturing flies, and this compound with Easy Trap and Olike were the worst combinations. In general, all combinations in both plots show two period of high fly captures: through the month of September and on mid October. In the other hand, captures of natural enemies in traps were also counted and specially for *Chrysopa sp* individuals, a very common and important natural enemy on olive groves. The best trap in capturing flies was also one of the traps with a low ratio n° of *chrysopas*/ n° of *flies* (0'07 in both plots) and these data must be considered to determine the real effectiveness of the traps.

Conclusions: Mass trapping could be an interesting method to be applied against the olive fruit fly in the Valencian Community but it must be analyzed not only the effectiveness of the trap but also its cost and its effect on natural enemies in order to select the best one.

Keywords: Olive fruit fly, traps and attractants, mass trapping.

LABORATORY EVALUATION OF THE EFFECTS OF ATTRACT & KILL FORMULATIONS ON *ANASTREPHA FRATERCULUS*.

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Background: A new attract & kill (A&K) formulation was developed by ISCA Tecnologias to control fruit flies. In laboratory tests, we compared the efficacy of this new formulation with three commercial products used by fruit growers in southern Brazil. Mortality tests were performed with *Anastrepha fraterculus* (Diptera: Tephritidae) and results showed that the effectiveness of Anarosa formulations was directly proportional to increasing dose of insecticide. Formulations containing organophosphates and cypermethrin produced similar mortality results. The Anarosa formulations, regardless of the insecticide dose, were more effective than GF 120 for *A. fraterculus* control.

Methods: Experiments were conducted at the Entomology Laboratory of EMBRAPA Grapes & Wine, Bento Gonçalves, RS (temperature 25°C±2, relative humidity (RH%) 70±10, photoperiod 14 hours) with insects reared on a diet of wheat germ, brown sugar and soy extract (3:1:1). Mortality of adult 15-20 days of age *A. fraterculus* was evaluated. Each replicate consisted of a cage (300ml plastic cup without bottom, containing a circular floor paper, a 2 ml container for water and 0.8g of solid diet) with two pairs of adult insects. Treatments were applied to leaves of *Vitis vinifera* plants in a greenhouse setting. Using disposable syringes, a 5ml drop (4 mm in diameter) was placed on the upper leaf surface. After a three hour drying period, the leaves were collected in the laboratory and cut into a circular shape (3cm diameter) containing a drop of the treatment which was then presented to the adult flies. Each treatment was replicated 13 times; each cage was a replicate. Measurements were taken every two hours for the first 24 hours, followed by intervals of four hours until the completion of assessments after 96 hours.

Results: The most rapid mortality resulted from the Anarosa + 0.2% cypermethrin and Biofruit + 0.15% malathion treatments, which caused 100% mortality after 36 and 32 hours respectively. Anarosa + 0.4% spinosad resulted in 100% mortality after 44 hours. The Anarosa + 0.2% spinosad and molasses + 0.15% malathion treatments both resulted in 100% mortality after 48 hours. For the Anarosa treatments containing 0.1% and 0.05% spinosad, 100% mortality was reached after 52 and 60 hours for each treatment respectively. The GF 120 treatment resulted in 96% mortality at the end of the tests, while the standard treatment, Anarosa without insecticide, resulted in 13% mortality at the end of the experiment.

Conclusion: Anarosa treatments proved to be promising for controlling populations of *Anastrepha fraterculus* and the mortality effect of the formulations progressively increases with increasing insecticide concentration

Keywords: Anarosa, Spinosad, Mortality, *Anastrepha fraterculus*

PERSISTENCE OF ATTRACT & KILL FORMULATIONS OF *ANASTREPHA FRATERCULUS*: GREENHOUSE AND RAIN-FAST EXPERIMENTS.

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Background: Food attractants associated with pesticides have been used as a strategy for management of fruit flies in fruit growing areas of Southern Brazil. Commercial formulations require frequent applications due to environmental factors like sunlight and rain. Anarosa, developed by ISCA Technologies, was evaluated for persistence after exposure to solar radiation and rainfall in laboratory tests on mortality of adult *Anastrepha fraterculus*.

Material and Methods: Experiments were conducted to evaluate the persistence of two attract and kill (A&K) formulations of Anarosa containing 0.1 or 0.2% Spinosad, compared to two standard treatments, GF 120 (0.02% Success® CB) and hydrolyzed protein (Biofruit®) + malathion on adult *A. fraterculus*. Experiments were conducted on *Vitis vinifera* in a greenhouse setting. Five plants were used per treatment. Treatments were applied as droplets (4mm diameter, 20 droplets) to upper leaves using disposable syringes (5ml). After 1, 7, 14, 21, 28 and 35 days after application, leaves with treatments were collected for rain-fast testing. Each treatment was exposed to 20 and 50mm of artificial rain; then leaf samples were collected and presented to flies in the laboratory to measure mortality. Each treatment was composed of 13 replicates consisting of a cage (300ml plastic cup without bottom, containing a circular floor paper, a 2ml container for water and 0.8g of solid diet) with two pairs of adult insects. For each evaluation, leaves with treatment droplets were cut in a circular shape (3cm diameter) and presented to the insects. Measurements were taken every two hours for the first 24 hours, followed by every four hours until the completion of assessments after 96 hours.

Results: Samples exposed to 20 mm of simulated rainfall resulted in 75 and 83% mortality after 96 hours for Anarosa 0.1 and 0.2% Spinosad treatments, respectively whereas GF 120 + malathion and Biofruit + malathion each resulted in only 39 and 18% mortality after 96 hours. The control sample resulted in 4% mortality. Samples exposed to 50 mm of simulated rainfall resulted in 59 and 83% mortality for Anarosa 0.1 and 0.2% spinosad treatments, 9 and 4% for the GF 120 + malathion and Biofruit + malathion treatments and 4% for the control treatment. Samples exposed to solar radiation showed no significant differences in mortality after 1 to 21 days with all resulting in 100% mortality.

Conclusions: The persistence of both the Anarosa and GF 120 formulations was similar after exposure to solar radiation, however the Anarosa formulation proved highly persistent with respect to rain, maintaining efficiency after considerable rainfall volumes.

Keywords: South American fruit fly, rain-fast, solar radiation, Anarosa, GF 120

FRUIT INFESTATION ON SOME CUCURBITS AS INFLUENCED BY PUPAL DENSITY OF MELON FLY, *BACTOCERA CUCURBITAE* (COQUILLET) IN SOIL AND THEIR MANAGEMENT THROUGH SOIL TREATMENTS.

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Background: Melon fly, *Bactocera cucurbitae* (Coquillett) is the most notorious pest of fruit vegetables causing considerable damage worldwide. This biotic pressure is increasing day by day due to intensive cultivation of HYVs under chemicalized agriculture, which leads to several environmental ill effects. Therefore, a sustainable management technology is a dire need of the hour. Here a novel eco-friendly approach has been made to combat this pest thorough estimation of pupal density in soil, its impact on percent fruit infestation and evaluation of some pesticide molecules including microbial.

Methods: Four important cucurbitaceous vegetables in West Bengal, India viz. pumpkin, *Cucurbita moschata* L., bittergourd, *Momordica charantia* L., ridgegourd, *Luffa acutangula* Roxb. and cucumber, *Cucumis sativus* L. were selected and the fruits were observed for melon fly infestation at weekly interval. Subsequently from each field soil samples below 12 cm depth were collected for pupal count. For evaluation of residual toxicity of some chemicals, soil was collected from experimental plot and after oven drying the same was kept in a plastic container followed by insecticide application. The third instar maggots at 'jumping stage' were released in the test container after 1, 3, 5 and 7 days of application of insecticides. The data thus obtained on various stages of development were subjected to statistical analysis by appropriate techniques.

Results: Highly significant positive correlations were established between pupal density and fruit infestation with having correlation coefficient 'r' = 0.984, 0.987, 0.962 and 0.923 on pumpkin (*Cucurbita moscheta* L.), bittergourd (*Momordica charantia* L.), ridgegourd (*Luffa acutangulata* Roxb.) and cucumber (*Cucumis sativus* L.) respectively. Economic threshold level (ETL) worked out from the individual regression model showed 0.47, 0.24, 0.53 and 0.56 adult male per trap per day for the respective cucurbit vegetables. Among the different chemicals significant differences in pupal mortality, pupal deformity and adult emergence were observed. Soil application of dichlorvos 76 EC emerged as most effective with highest mean pupal mortality (63.33%) and lowest mean adult emergence (10%) followed by carbofuran 3G (58.33, 12.17%) and chlorpyrifos 20EC (56.25, 15.42%). But bleaching powder and formaldehyde (4%) proved to have insignificant effect on adult eclosion as 80.33 and 76.33% adult already eclosed from bleaching powder and formaldehyde treated soil respectively. Whereas maximum deformed pupae (28.75%) was recorded from the soil treated with the microbial biopesticide, *Metarhizium anisopliae* (1.15WP).

Conclusions: The above findings established that the percent fruit infestation is highly influenced by its pupal density in field soil and their different developmental stages could be disrupted through soil application of chemicals. Also suggest that it could be an efficient tool for sustainable management of melon fruit fly before their emergence.

Keywords: *Bactocera cucurbitae*, fruit infestation, pupal density, mortality, deformity, eclosion, soil treatments

OLIVE FRUIT FLY, *BACTROCERA OLEAE* GMEL., MASS-TRAPPING WITH OLIFE TRAPS: EFFECT OF HOLE SIZE IN THE FLY AND NON-TARGET ARTHROPODS CAPTURES.

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Background: The olive fruit fly, *Bactrocera oleae* Gmelin (Diptera: Tephritidae), is a key-pest of olive tree in the Mediterranean region. In organic agriculture, the use of Olife traps for olive fruit fly mass-trapping is currently used in different regions of the Iberian Peninsula. However, previous works have demonstrated that trap hole size influences the number of fly and non-target arthropods captures, that could have a significant impact of beneficial arthropods communities. In this context, the present work aimed to study these aspects as well as the efficacy of the method in the control of the pest.

Methods: The work was conducted in an organic olive grove located in Mirandela (Northeast of Portugal). The olive trees have medium size and belong to Cobrançosa variety. Four treatments (bottle hole sizes) were selected (4, 6, 8 and 10 mm), and each tested in one hectare orchard at basis of one trap per tree. In each treatment with a bi-weekly basis 15 traps were changed and the number of flies and no-target arthropods were counted. The attack of fruits was observed in 25 fruits per tree in a total of 20 trees.

Results: In all treatments a total of 8922 individuals were counted and ants were the major group recovered. A marked effect was observed in the total number of non-target arthropods if ants are excluded. The use of these bottles was particularly harmful to the adults of the Chrysopidae family that represents between 3.7% and 17.6% of the total recovered (excluding ants). Concerning to *B. oleae*, a reduced number of adults were observed in all treatments. No differences were registered in the attacked fruits between bottle hole size. And, the fruit attack reaching 19% in the control plot (without traps) that was about the double of treated plots.

Keywords: Olive fruit fly, Olife traps, hole size, no target arthropods, organic agriculture

MEDFLY CONTROL IN MEDITERRANEAN AREAS OF SPAIN WITH M3 TRAPS.

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Background: The Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), is a key-pest of fruit trees in the Mediterranean region. Lure and kill traps as M3 represent an efficient system to control fruit flies. Several trials are presented in this work to ascertain its efficiency under different mediterranean conditions and in different crops.

Methods: The work was conducted in several citrus and stone fruits orchards Bétera, Vilavella, Sagunto, Pobra de Farnals, Alama de Murcia, Jumilla, Yecla and Lérida. Traps were hold in place at 360 traps per ha, at 45 days prior fruit harvest. Fruit damage was evaluated in all orchards. Control traps baited with food attractants were placed in the orchard perimeter and one in the center.

Results: In all the assays a significant fruit damage reduction has been obtained when compared to control orchards subjected to chemical control. Its broad application for organic agriculture is discussed.

Keywords: M3 traps, fruit damage, organic agriculture

EFFECTIVE SAMPLING RANGE FOR PROTEIN-BASED LURES.

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Background: Effective sampling range is defined as the maximum distance from which an insect can reach an attractive source in a given period of time. Information on sampling range is valuable for determining appropriate coverage of traps for use in population delimitation, mass trapping control strategies, or identification of foci of infestation for precision targeting of control measures. Despite extensive use of protein-based female-targeted attractants in current trapping programs for tropical tephritids, there is little documentation their effective sampling range.

Methods: Studies were conducted in Florida and Honduras. A geostatistical analysis approach, using data from capture of wild flies in a specifically designed trapping grid, was compared with a standard release/recapture study approach that used laboratory-reared sterile and wild flies (Florida), and recently captured wild flies (Honduras) to determine effective sampling range. Field design consisted of 38 traps (over 0.5 ha) placed in a combination of standard and high density grids to facilitate geostatistical analysis, and tests were conducted in coffee, mango and orthonique with the three component lure (APT: ammonium acetate, putrescine, trimethylamine, Honduras) and guava with the two component lure (AP) and torula yeast/borax (TY, Florida).

Results: Effective sampling range, as determined by geostatistical analysis for APT for *C. capitata*, was ~30 m for flies captured in coffee or mango and ~ 40 m for flies captured in orthonique. Similar results were obtained from the release/recapture study, and contour maps indicated that wind direction had a strong influence on sampling range, which was ~15 m greater upwind as compared to downwind from the release point. In release/recapture tests with mature wild *A. suspensa*, range of AP was determined to be 30 m. With sterile females, effective range of both AP and TYB was 20 m. Sterile immature *A. suspensa* females dispersed farther and were recovered in higher numbers than mature females, and recapture with AP was higher than recapture with TY.

Conclusions: Geostatistical analysis of field-captured insects in appropriately designed trapping grids may provide a supplement or alternative to release/recapture studies to estimate sampling ranges for semiochemical-based trapping systems. Contour maps indicated that wind direction had a strong influence on the active space of attractants, as reflected by distribution of captured flies in release/recapture studies.

Keywords: *Ceratitidis capitata*, *Anastrepha suspensa*, synthetic lures, torula yeast

FIELD ASSAYS OF NEW CONTROLLED-RELEASE PHEROMONE DISPENSERS FOR CONTROLLING *BACTROCERA OLEAE* (GMELIN).

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Background: The eco-trap systems for controlling the olive fly, *Bactrocera oleae* (Diptera: Tephritidae), are used worldwide in integrated pest management programmes. The low biodegradability of most commercial pheromone dispensers requires finding other alternatives more ecological to the currently existing ones. We report here on several type of eco-friendly, hand-applied pheromone (1.7-dioxaspiro [5.5] undecane) dispensers tested in both laboratory and field.

Methods: Clay materials and different additives, in changing proportions, were designed and evaluated in order to obtain that possessing the optimal pheromone emission. The effectiveness of the best formulations were evaluated in field and subjected in parallel to a procedure of accelerated aging in a temperature and wind speed controlled chamber. Residual pheromone remaining in the dispensers was periodically evaluated by gas-liquid chromatography. Release rates and half-life times were determined.

Results: Two formulations (four types of dispensers) showed the best performances in the laboratory as compared with the commercial *B. oleae* Long Life Lure from Agrisense[®] (Pontypridd, UK) so they were finally tested in the field. Design 1E Type showed the best behaviour in the field with an average controlled release of emission of 0.6 mg pheromone/dispenser.day, from day 21, which is slightly higher than that showed by the commercial one. In addition, it showed an initial loss of pheromone 10% lower than that showed by Long Life Lure. Maximum differences in emission rates of all dispensers ranged between 0.1-0.9 mg pheromone/day. The average of captures obtained in SEVEP[®] traps (EPA, SP) were not statistically significant despite the differences in emission found among dispensers. The best half-life time obtained was higher than 4 months, which allows to dispenser to be placed in the field without replacement during an entire olive fly campaign in the Mediterranean area of Spain.

Conclusions: The field emission tests of dispensers designed confirmed the good performance obtained in the laboratory. The mean of captures were as effective as the best dispenser on the market, which combined with their easy biodegradability make to be suitable for monitoring and controlling *B. oleae*, especially in "Lure and kill" programs.

Keywords: Olive fly, dispenser, pheromone, monitoring, control.

STRUCTURAL ABNORMALITIES IN THE EGGS OF FRUIT FLY, *BACTROCERA TAU* (WALKER) AFTER FEEDING ON PHLOROGLUCINOL.

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Background: Fruit fly, *Bactrocera tau* is a major pest of tomato and cucurbit vegetables and loads of insecticides are used to prevent the damage by this fly but in the process the fruit gets poisoned making the consumption unsafe. Phloroglucinol (1,3,5 trihydroxy benzene) has been reported as an egg shell peroxidase inhibitor and here in the present study we report the effect of this novel chemical on the eggs of *B. tau* when fed on artificial diet containing this chemical, under laboratory conditions.

Methods: In the first set of experiment, five pairs of freshly emerged adults of *B. tau* were given a diet containing glucose, protein hydrolysate and water (4:1:5) provided with different concentrations of phloroglucinol (1,5 and 10mM (milli Molar) in first subset, and 25, 50 and 100 mM in second subset). These fruit flies were provided with a host (cucumber) for egg laying from 9th day onwards. In the second set of experiment, 8 day old flies were provided with phloroglucinol containing diet and the host was provide for egg laying as in the first set. Observations on egg laying and the structure of eggs were taken.

Results: In Set-1, where the adults were fed continuously on phloroglucinol containing diet, normal egg laying was recorded at 1, 5 and 10mM phloroglucinol concentrations. Egg count was slightly less (158.0eggs/female) at 25mM concentration in comparison to 187.0 eggs/female in control. However at 50 and 100mM concentrations of phloroglucinol the egg laying reduced drastically to 26.2 and 12.0 eggs/female, respectively. Further, the eggs laid were fragile, very thin or with ruptured chorion at 50mM, whereas at 100mM in addition some chorionless eggs or vitelline membrane bound oocytes (VMOs) were also laid. In Set-II where the diet containing phloroglucinol was provided after 8 days of emergence, a similar trend was recorded.

Conclusions: Phloroglucinol has a marked effect on egg development of *B. tau* particularly on egg chorion as it acted by inhibiting egg shell peroxidase when fed at 50 and 100mM concentrations. It resulted in eggs with thin chorion, ruptured chorion and vitelline membrane bound oocytes (VMOs), which failed to hatch. The study suggests that it can be an effective ecofriendly tool in IPM of *B. tau*.

Keywords: Fruit fly, *Bactrocera tau*, phloroglucinol

FRUIT FLIES SPECIES COMPOSITION AND SEASONALITY, AND RELATIVE EFFICIENCY OF FOUR FEMALE ATTRACTANTS IN MANGO ORCHARDS IN BENIN.

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Background: Trapping of tephritid fruit flies has received a great deal of attention for detection, monitoring and control purposes. Several commercially available female attractants have been developed and are widely used, but information on their usefulness in fruit fly monitoring under various environments in Africa is scarce. Here we report on species composition and seasonality of fruit flies caught in four commercial female attractants in mango orchards in Benin (West Africa).

Methods: The experiments were conducted in eight mango orchards in 2007 and 2008 and 12 orchards in 2009 – spread across four agroecologies in Benin. Orchards contained a range of mango cultivars representing what is grown in the targeted areas. Fruit fly trapping was carried out each year during the mango fruiting period – March through July. Four commercially available attractants were tested using Multilure traps. Three traps per attractant and per orchard were used. Torula yeast was used as the standard in all three years and was compared with Nulure in 2007, BioLure in 2008, and Mazoferm in 2009. Traps were serviced at weekly and biweekly intervals for liquid and dry attractants, respectively.

Results: Overall, eleven fruit fly species belonging to three genera were found in traps, with *Bactrocera invadens* and *Ceratitis cosyra* being the most abundant. Species composition in traps mirrored those in infested mango fruits, with species relative abundance depending on agroecology. *Bactrocera invadens* was present in all study sites but its seasonality followed a pattern of dominance throughout the mango season in the south while being nearly absent in the drier agroecologies in northern Benin during the first half of the season, but its abundance increased steadily in central and northern Benin with the onset of rains and became the dominant species during the second half of the season. *Ceratitis cosyra* abundance followed patterns opposite to those of *B. invadens*. Torula yeast attracted considerably more fruit flies than BioLure (20% of Torula yeast) regardless of agroecology ($P < 0.05$), but attracted similar numbers of flies as Mazoferm, except in the Sudan Savanna where Torula yeast attracted more *B. invadens* ($P = 0.008$) and *Ceratitis* spp. ($P = 0.048$) than Mazoferm. Nulure was generally less attractive to all fruit flies than Torula yeast.

Conclusions: The study revealed that Torula yeast and Mazoferm are equally attractive to the fruit fly species assemblage in mango orchards in Benin, followed by Nulure and BioLure. There were not any significant differences; however, in relative attractiveness of the four attractants to *B. invadens* and *Ceratitis* spp. Implications for fruit fly monitoring and suppression are discussed.

Keywords: Multilure traps, protein bait, Torula yeast, Nulure, BioLure, Mazoferm.

ORGANOPHOSPHATE RESISTANCE IN ORIENTAL FRUIT FLY MAY AFFECT THE ATTRACTING EFFICIENCY OF OP-BASED INTOXICANT LURE.

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Background: The oriental fruit fly (*Bactrocera dorsalis* (Hendel)) is an economic pest of great significance in the Asia-Pacific region. The most common management tactic is the male annihilation technique (MAT), which consists of using highly attractive toxic lures. The toxicant used in these lures are most often organophosphate (OP) based insecticides. In recent years, fruit flies in Taiwan have been reported to have OP resistance in the field. Similar cases of resistance development and subsequent effectiveness reductions to OP based insecticides have been observed in a wide range of alternate fly species in different localities. However, at this time, there have been no evaluations concerning the control efficiency of OP-based intoxicant lures when resistance is exhibited in the field.

Methods: The attracting efficiency of naled with methyl eugenol (ME) to two different levels of OP-resistant and one susceptible male oriental fruit fly lines were evaluated under greenhouse conditions in Taiwan. Two concentrations of naled (5 and 2.5%) with 90% of ME were used to evaluate the attraction efficiency within the traps, or cages, after 1 h, 24 and 48 h post-treatment. Flies from each of the 3 lines were placed inside a perforated plastic fruit fly trap, and then the traps were suspended by means of a wire attached to the roof of a organdy 1 m³ cage.

Results: The attracting efficiency of *B. dorsalis* males in the three lines were higher for the 5% naled treatments. Of the three lines, attraction was highest in the higher OP-resistant line, followed by the lower OP-resistant line, and the least efficiency was exhibited in the susceptible line at different posttreatments times.

Conclusion: In our study, OP-resistant lines affected the attracting efficiency of OP-based intoxicant lures. When applying MAT to oriental fruit fly control, the intoxicant resistance needs to be considered.

Keywords: *Bactrocera dorsalis*, MAT, naled, methyl eugenol.

IMPROVED FRUIT FLY LURES AND FORMULATIONS FOR DETECTION, DELIMITATION AND CONTROL.

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Background: Exotic fruit fly detection, delimitation and control using semiochemicals-based attractants form the foundation of area-wide pest management programs worldwide. Increasingly, countries require detection arrays as a basis for certification of fly-free areas, low prevalence areas and for development of systems approaches which all facilitate international trade. Improved attractants and formulations for key fruit fly pests such as *Ceratitis* sp., *Anastrepha* sp., as well as methyl eugenol and cue-lure responding *Bactrocera* are needed to improve detection, reduce costs and facilitate auditing of regulatory areas of concern. We report here on recent developments to improve lures and formulations for use in fruit fly programs.

Methods: New (improved) attractants have been developed for *Ceratitis* (ceralure B1), methyl eugenol-responding *Bactrocera* (Iodo-eugenol) and cue-lure responding *Bactrocera* (melolure and cuc-lure). Attractants were identified, formulated and tested in the field against representative species and in some cases multiple species in conjunction with collaborators. Data were collected in a variety of bioassay arenas including flight tunnels, field cages and in open field tests lasting up to 10 weeks or more. Additionally, several new and/or improved formulations of existing attractant lures have been developed that reduce handling of liquid lures on cotton wicks and include toxicants in the formulations.

Results: Many of the new attractants were shown to be more attractive than their predecessors based on molecule to molecule comparisons (ceralure B1 or melolure), or showed increased attractancy and female bias relative to proteinaceous baits (cuc-lure). These new attractants have also been tested in newer formulations such as solid matrix composites that allow the lure to volatilize at a more constant rate than the cotton wicks. Of the various toxicants tested, incorporation of naled or DDVP into the solid matrix as been effective, while reduced-risk insecticides such as spinosad or rynoxypur have had success with methyl eugenol containing formulations but less so with cue-lure formulations under field conditions.

Conclusions: New and improved lures and formulations of fruit fly attractants have improved usability and detection capabilities for several tephritid species. These formulations should be considered by action agencies and regulatory bodies for inclusion into standardized detection grids or for control technologies such as mass-trapping, male annihilation and bait sprays.

Keywords: Tephritidae, *Bactrocera*, *Ceratitis*, trapping, detection, control.

IMPACT OF THE PROJECT ON INTEGRATED MANAGEMENT OF FRUIT FLIES IN INDIA (IMFFI) IN FRUITS AND VEGETABLES CULTIVATION IN SOUTHERN KERALA.

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Background: Oriental fruit fly, *Bactrocera dorsalis* Hendel and melon fly *B. cucurbitae* Coq. are serious pests of mango and cucurbits, respectively. *B. dorsalis* is a direct pest on mango. In India, the loss in fruit yield ranges from 1 to 31% (Verghese *et al.*, 2002). In vegetables the extent of crop loss due to *B. cucurbitae* varies between 30 to 100 % (Sookar *et al.*, 2006). The adult female lays eggs in maturing fruits and vegetables. The maggot develops in the fruit, causing drop and rotting. ICAR–UKDFID project on integrated management of fruit flies in India (IMFFI), which was in operation during 2002-2005 came out with packages for fruit fly management in India both for *B. dorsalis* and *B. cucurbitae*. The objective of the present study was to assess the impact of the research results of IMFFI in reducing the crop loss due to fruit fly attack in mango and cucurbitaceous vegetables in southern Kerala [south India]. The study also assessed the feedback of farmers on the effectiveness of this technology and the scope of its commercial adoption.

Methods: Trials in three southern districts of Kerala were carried out in farmers' fields, maintaining demonstration plots, during April-June, 2009 to evaluate the efficacy of integrated pest management involving MAT using methyl eugenol traps/cue lure traps and Bait application technique (BAT), using food baits for the management of fruit flies infesting mango and snake gourd. Field sanitation and biocontrol methods were also integrated. Adoption of the technology was also assessed, based on feed back.

Results: There was significant reduction in the level of *B. dorsalis* infestation in farmers' fields where IPM was followed. In mango, in the three districts of Thiruvananthapuram, Kollam and Alappuzha the control plots recorded an infestation level of 80.41, 80.89 and 83.38 % as against the treated plots with a pest incidence of 8.41, 7.6 and 8.24 %, respectively. In snakegourd in the three districts Thiruvananthapuram, Kollam and Alappuzha, the control plots recorded an infestation level of 73.74, 73.16 and 74% as against the treated plots with a pest incidence of 7.4, 7.77 and 4.99%, respectively. The results are in conformity with the finding of the ICAR-UK DFID project on fruit flies. *Beauveria bassiana* owing to high infectivity and easiness of application gave encouraging results for the management of fruit flies..

Conclusions: The application of biocontrol agent can effectively be incorporated as a component of integrated fruit fly management programme in mango and vegetable crops. The feedback was encouraging, which authenticates the findings of the IMFFI Project

Keywords: IPM, IMFFI, *B. dorsalis* , *B. cucurbitae*, snake gourd.

SIDE EFFECTS OF SELECTED PESTICIDES COMMONLY USED IN CITRUS ORCHARDS ON STERILE MEDFLY MALES.

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Background: The Sterile Insect Technique (SIT), can be considered a biological control method, as it is based on rearing, sterilizing and releasing large numbers of males to mate with wild females, who will then produce unviable eggs. Hence, sterile medfly males can be considered as beneficial organisms within an integrated pest management program. Sterile males released may directly be exposed to pesticides used against other citrus pests in the target area. Therefore, the side-effects of these pesticides should be assessed on these males.

Methods: Laboratory trials were conducted to find out side-effects of the pesticides abamectin, chlorpyrifos, etofenprox, etoxazole, narrow-range mineral oil, spinosad, pymetrozine and pyriproxyfen on Vienna-8 *tsl* sterile *C. capitata* males. Three-day-old sterile males were exposed to fresh, 7, 14 and 21-day-old residues of the selected pesticides. Mortality of sterile males was observed daily during 6 days after pesticide application. When differences against the control were found, the resulting corrected mortalities (Abbott) were interpreted according to IOBC standards.

Results: All pesticides resulted harmless to Vienna-8 *tsl* sterile males except for chlorpyrifos and spinosad. Both pesticides resulted moderate harmful according to the IOBC categories for the fresh residue and decreased to slightly harmful until residue of 14-d-old. At 21-d-old residue, chlorpyrifos resulted harmless whereas spinosad continued as slightly harmful.

Conclusions: Our results provide useful information to implement an SIT program within an citrus area where an IPM program is being conducted. Sub-lethal effects of pesticides on sterile Vienna-8 strain of *C. capitata* males mating behavior should also be considered for a complete analysis of their impact.

Keywords: *Ceratitis capitata*, sterile insect technique, insecticide toxicity.

FIELD EVALUATION OF GF-120 TO CONTROL *CERATITIS CAPITATA* (WIEDEMANN) IN IRAQ.

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Background : The medfly, *Ceratites capitata* has been recorded for the first time infested orchard citrus fruits in late 2006, and since then spread to all areas of orange grooves and become one of the major pest in Iraq. This because of citrus fruit importation from neighboring countries known to have this pest for decades and for inefficient national quarantine system. The crises depicted the urgent need for the implementation of national control campaign, taking in mind using effective alternatives to harsh insecticides with low environmental risk. GF-120 consist of spinosad as an active ingredient, derived from bacteria, and mixed with attractant and feeding stimulant agents, proven to be effective in controlling many pest species including medfly with a low mammalian and wildlife toxicity.

Materials & Methods : The efficacy of GF-120 (Spintor Fly 0.24 g/l, Dow AgroSciences) was evaluated against *C. capitata* in citrus orchards of Iraq. Trials were carried out in mandarin oranges orchard at Baghdad in the year of 2007, and in naval, mandarin and sour orange orchard at Wasset province (50 km east of Baghdad) in the year of 2008. For all treatments FG-120 was applied as spot treatment in a concentration of 300ml/5 L water. Treatment of 2007, the solution was applied two times with 10 days interval using 15 L handbach spryer. Twenty five trees (replicates) were treated each time, in addition to 10 trees for the control. Differences between treatments were determined by checking, at 10 days after treatment and at harvest time, 100 fruits randomly picked. Treatment of 2008 was applied at three times: before adult emergence, after adult emergence and after 10 days of the 2nd treatment. The experiments were designed to have four groups of trees, each with 25 trees and the control. The 1st group treated 3 times, the 2nd treated twice, the 3rd group treated once and the control group. At the end of the season, percentages of infested fruits were determined by checking of 100 fruits for each group.

Results: The result of the experiments carried out in 2007 on orange mandarin indicated lower percentage of damaged fruits (5-8%) in treatment with GF-120 compared with untreated check (21-36%). In the trial of 2008, on mixed oranges treatment resulted statistically different between treatment timing and number. Percentage of infestation achieved were 3,5,10% for 3,2 and one time treatments ,respectively. All treatments result showed significant difference compared to the untreated check (89%).

Conclusion: Results achieved in all trails showed that spot foliar surface treatment with GF-120 is a promising product to control the medfly, *C. capitata* with low impact on the environment.

POPULATION MONITORING OF *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE) IN S. MIGUEL ISLAND IN THE LAST FIVE YEARS.

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Background: To develop a control program against the Mediterranean fruit fly, or medfly, *Ceratitidis capitata* (Wiedemann), is essential to know the population size and presence around the year to establish the programs. Population monitoring is a key point in control programs. The extent of lures and traps available in the market pushed us to establish a pilot program to select the best trap and lure for the San Miguel Island.

Methods: Monitoring was conducted through 2005-2009 in three fruit orchards in the central part of São Miguel Island, Azores. One orchard was a monoculture orchard and the other two were mixed-fruit orchards. Monitoring used “Easy-trap”, with two different attractants: the male specific pheromone trimedlure and a food-based attractant – “Ferag” lure (ammonium acetate + diaminoalcane + trimethylamine). Two traps, one with each attractant, were placed in each orchard. Traps were suspended in the tree canopy.

Results & Conclusions: In the two mixed-fruit orchard adults were captured year-round with a break during March-May. In the monoculture orchard, however, captures were limited to July-December. Significant differences were observed between the two types of attractants and among the years, independently of the location of the orchard. The mean number of adults per day captured in the pheromone trap was significantly higher than traps baited with Ferag lure. However, using both attractants increases the potential of controlling the fly infestations by better predicting the presence and the numbers of the flies.

Keywords: Med Fly, monitoring, attractants

ECONOMIC IMPORTANCE OF THE MEDITERRANEAN FRUITFLY, *CERATITIS CAPITATA* IN THE ORCHARDS OF CENTRAL IRAQ.

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Background: In the recent years, The Mediterranean Fruitfly *Ceratitidis capitata* is distributed in the orchards of central Iraq, and caused highly economic losses.

Methods: The study was conducted in orchards in central Iraq during 2009 and make field survey of the insect in four types of orchards (citrus, Apricot, Figs & Citrus, a mixture of fruit trees), and used for this purpose Tefri traps supplied with Q-lure and DDVP.

Results: The results of the study for the presence of the insect throughout the year ,and reached its highest numerical density of the insect in citrus orchards during the month of October and November were (295 – 870) insect per trap per 2 week and the least numerical density during the months of January and February .While the numerical density of the insect in the orchards of Apricot ,Figs & Citrus ,a mixture of fruit trees were 9 ,166 , 207 Insect per trap per 2 week each respectively, during the months of October and November.

Conclusions: The *C. capitata* caused highly economic losses in citrus orchards reached 60% of the mandarin fruits .Currently in Iraq to fight no control method to reduce the economic losses caused by this pest except the use of pesticides GF-120.

Keywords: Fruitfly , Survey , Iraq

ELECTROANTENNOGRAPHY FOR EVALUATION OF FOOD-BASED ATTRACTANTS FOR PEST TEPHRITIDAE.

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Background: *Anastrepha* fruit flies are serious economic pests of fruit crops throughout the American tropics and subtropics. Current trapping systems utilize synthetic lures that emit ammonia and other attractant chemicals that function as protein feeding cues. However, fly captures are variable with synthetic lures, and this variability does not appear to be correlated solely with ammonia release rate. The need for improved attractants prompted research using electroantennography (EAG), a technique that measures response of antennal olfactory receptors to volatile chemical stimuli.

Methods: All EAG analyses were conducted with a Syntech system (Hilversum, The Netherlands) using laboratory-reared adults (of known age and physiological state) of the Caribbean fruit fly, *Anastrepha suspensa* (Loew). Antennae were presented with quantified chemical samples in saturated vapor form, and EAG responses were recorded and normalized relative to a standard reference chemical (20 μ l 2-butanone).

Results: To date, our group has quantified antennal response of male and female *A. suspensa* to ammonia, carbon dioxide, ammonium bicarbonate, 2-butanone, 3-methyl-1-butanol, putrescine, cadaverine, and homologous terminal diamines. Analysis of the amplitude of EAG response has provided information on appropriate doses and combinations of chemicals needed to elicit optimal antennal response. Comparative EAG has identified several factors that contribute to the variability in fly response to known food-based attractants, including sex, age, nutritional requirements, and reproductive status. In addition, EAG has provided insight into the antennal chemoreceptors involved in detection of semiochemicals, and has identified potential new fruit fly attractants.

Conclusions: Quantitative electroantennography is a valuable tool for evaluation of peripheral olfactory response of tephritid flies to potential attractants. Information obtained from EAG analysis will be used to determine relationships between antennal sensitivity to food-based attractants and efficacy of those compounds when deployed in traps for capture of pest species.

Keywords: *Anastrepha suspensa*, electroantennography, olfaction, synthetic lures, food-based attractants

DEVELOPMENT OF SPLAT MAT CONTROLLED RELEASE SEMIOCHEMICAL BAIT FORMULATIONS FOR LONG LASTING FRUIT FLY MANAGEMENT

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Background: The insecticide landscape is changing rapidly in orchard crop production in the US and abroad. This is particularly true for nursery, greenhouse, fruit, olive and nut production where conventional insecticide products that form the foundation of current management programs are being lost. Small growers in particular, suffer with this increasing lack of available control tools and from pressure of suburban neighbors to reduce use of conventional insecticides. Current Male Annihilation Techniques (MAT) combine male-specific attractants with insecticide in traps and devices that, while effective, require routine service that is costly and labor intensive. ISCA Technologies' Specialized Pheromone and Lure Application Technology (SPLAT) was initially developed for the mechanical deployment of small doses of Lepidopteran pheromones for long lasting mating disruption. Here we report further development of this biologically inert matrix to create Tephritid management formulations that are hand or mechanically applied, rain-fast, and provide long-term controlled release of a variety of semiochemicals and pesticides at biologically relevant levels.

Methods: SPLAT was formulated with spinosad, malathion, naled and other insecticides along with attractants such as methyl eugenol, trimedlure, cue-lure, raspberry ketones, and other parapheromones, attractants and feeding stimulants to achieve specific killing action against a variety of Tephritidae, in both laboratory and field trials. Studies have been conducted to determine the longevity and mortality of SPLAT formulations for fruit flies resulting from variable point source densities, sizes and concentrations of active ingredients. Methods of SPLAT application ranged from hand-held caulking tubes and electric grease guns to mechanical and aerial applications.

Results: Field trials conducted in Hawaii showed that SPLAT could be combined with naled and spinosad, resulting in significantly higher mortality of *B. dorsalis* males with greater longevity than other baits and thickener formulations. Field trials in tropical urban areas indicate that a single application of SPLAT combined with methyl eugenol and Spinosad effectively controls *B. carambolae* for months. Studies with *A. ludens*, *C. capitata* and *A. fraterculus* have shown that SPLAT combined with spinosad, feeding stimulants and attractants resulted in up to 99% mortality within hours. Laboratory data also show that SPLAT is amenable to the inclusion of a wide range of active ingredients, including volatile alcohols, acetates and aldehydes which can increase attraction for some fruit fly species. SPLAT matrix provided protection of active ingredients tested and increased longevity, a particularly important issue for areas with high temperatures and rainfall.

Conclusions: Due to its amorphous and flowable quality, SPLAT MAT provides a mechanized attract and kill formulation for long-term farm-wide as well as area-wide management and control of populations of a variety of Tephritid fruit fly species. The size and density of point sources can be tailored to treat any sized field or pest density.

Keywords: SPLAT, Spinosad, Attract-and-kill, Attractant, Mortality

MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA* (WIEDEMANN) MASS-TRAPPING ON CLEMENTINE GROVES IN SPAIN: OPTIMIZING TRAP DENSITY.

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Background: In citrus, *C. capitata* mainly causes damage to early ripening varieties, such as Clementine (*Citrus reticulata* Blanco). Up to 8 sprays can be performed in a grove to control Medfly in a season. The mass trapping strategy attempts to provide a successful pest control. The system is based on placing a high amount of traps with an attractant and a toxicant, aiming to capture as large as possible number of adults in the field. In Spain, more than 30,000 ha of citrus groves protected against Medfly by mass trapping showed good efficacy using a density of 50 traps per ha during 3 months before harvesting. Our aim is to study the efficacy of this method specifically on the most abundant Clementine variety and optimize the trap density required to successfully control this pest.

Methods: Six commercial groves of Clemenules (*Citrus reticulata* Blanco cv. Clemenules) citrus variety of approximately 3 ha in size were divided each one into three plots and each plot was assigned a different trap density: 25 (D25), 50 (D50) and 75 (D75) traps per ha. Probodelt[®] (Maxitrap[®] model) traps with the attractant Ferag CC D TM[®] (SEDQ), comprising three membrane dispensers of Trimethylamine, Ammonium acetate and Diaminoalkane, were placed in position. DDVP was used as toxicant. Colour rind index, internal maturity of fruits, adults captured in traps, and fruit damage were assessed.

Results: Fruits from all groves and years were ready to harvest at the end of October, according to commercial quality parameters required for trade export. The higher the trap density, the lower the number of adult captures per trap and day with, on average, 1.21 ± 0.13 , 0.27 ± 0.02 and 0.23 ± 0.02 for D25, D50 and D75, respectively. However, the number of adults captured per ha was similar for D50 and D75 trap densities, while both were significantly lower than D25. The average number of adults captured was 270.2 ± 69.9 , 123.3 ± 28.7 , and 152.3 ± 53.7 for D25, D50 and D75, respectively. For all the years and plots, the maximum percentage of fruits attacked was 1.5%. The number of attacked fruits was similar between plots. Damaged fruits on the perimeter rows were significantly higher than inside the plots.

Conclusions: Mass trapping is a valid stand-alone control method against *C. capitata* in varieties of Clementine that start maturing by October, and a 25 traps per ha density is sufficient to ensure successful control of this pest. Therefore, our findings imply that a mass trapping control program, using 25 traps per ha, can be implemented providing protection against med fly over 50% of production in our citrus producing area.

Keywords: mass trapping, trap density, fruit fly, citrus.

FRUIT FLY MANAGEMENT IN OLIVES WITH A NOVEL INNOVATIVE BIOLOGICAL SYSTEM (GF-120[®]).

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Background: The olive fruit fly *Bactrocera* (= *Dacus*) *oleae*, is the most destructive pest of olives. Crop losses, quantitative and qualitative, due to this pest can reach up to 100% for table cultivars and 80% of oil value if no control measures are taken. In the last forty years a variety of methods have been used to control this pest and protect against significant economic yield losses. Current control methods include full cover, protein-based bait sprays and SIT. Bait applications have proven to be much safer to the crop and environment than the full cover sprays. GF-120[®] (SPINTOR[®] CEBO, SUCCESS[®], SPINTOR[®] FLY) is a concentrated pre-mixed fruit fly bait containing a reduced risk toxicant to both mammals and non-target insects. Attractants in GF-120 have been optimized to control a plethora of the most damaging and invasive fruit fly species. GF-120[®] attracts and controls male and female tephritid fruit flies. It contains an optimized blend of feeding attractants and a green chemistry insecticide, spinosad; which is derived from a naturally occurring soil bacterium, *Saccharopolyspora spinosa*.

Methods: Field and laboratory studies have been conducted in olives, throughout the main olive producing countries aiming to evaluate the efficacy against olive fruit fly and the medfly, selectivity, attractiveness, residuality, potential risk of resistance and impact on non-target organisms of GF-120[®].

Results: GF-120[®] applied at the rate of 1.0 -1.6 L pr/ha as a true bait showed that it is very effective against olive fruit fly and the medfly compared to the traditional control programs consisting of tank mixes of organophosphate or pyrethroid with hydrolyzed protein. Optimized bait application technology targets achieving large droplet size (4-6 mm) which results in improved attractiveness and residuality. The potential risk of resistance development was evaluated in six consecutive generations and proved that it is very low. GF-120[®] impact on non-target organisms (NTOs) has been evaluated in a number of studies confirming greater levels of selectivity compared to traditional fruit fly control solutions (organophosphate or pyrethroid with hydrolyzed protein). GF-120[®] is approved for use in organic farming and has an excellent environmental profile and has been demonstrated under laboratory, and field conditions to be an ideal product for use in IPM programs and sterile insect technique (SIT) control strategies.

Conclusions : Years of extensive research trials and commercial use across the globe have proven that GF-120[®] applied either by ground or by air at 1.0-1.6 lt pr/ha targeting true bait applications provides the most effective, safe and organic solution for the olive industry.

Keywords: Spinosad, GF-120[®], *Bactrocera oleae*, Olive fruit fly, insecticidal baits.

COMPARATIVE STUDIES OF THE EFFECTIVENESS AND ATTRACTIVENESS OF THE TRAP "SENTRAP" AND TWO HOME-MADE PROTOTYPES TO "MULTILURE" (CONVENTIONAL TRAP) IN MALE ANNIHILATION TECHNIQUE.

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Background: If pests could be attracted to few points where they would be either in contact with or consume the toxin then many of the objections that confront broadcasted pesticides could be overcome. Studies have shown that peak population of *Bactrocera invadens*, the most destructive invading fruit fly species, occurs during the wettest periods with an abundance of food. So, such situation limits the use of killer-blocks in open air in Male Annihilation technique (MAT). This study aims to compare the effectiveness and attractiveness of these different local trapping devices to the conventional "Multilure" (Better World Manufacturing, Inc., Miami, FL) regarding partly the limited financial resources of producers.

Methods: The study was conducted in a 10-years-old mango orchard of fifty six (56) ha of the late season variety "Kent" in Senegal. It was set in 3 randomized blocs and treatment repeated 4 times with 50m between traps. Killer-blocks (5 x 5 x 1,25 cm) of Triplex are soaked in Mal'atrap (75% methyl eugenol and 25% malathion). Flies were collected separately every 10 days. The experiment lasted from July 15 to October 15, 2008. Data variability on individuals were analyzed using a one-way ANOVA, means separation between treatments performed by the Tukey honestly significant difference (HSD) test at 5%. The dependence with variables such as time and catch was done using the test of Pearson correlation.

Results: Average catches of "Multilure" to those of 1.5-liter, "Sentrap" and 5-liters showed a significant difference ($F = 5.472$, $df 3$, $\alpha = 0.003$). However, Results from the Tukey HSD test indicated that only 5-liters trap was statistically comparable to "Multilure". The study of the relationship between catch at different times and type of trap showed strong relationships. Sentrap, and "Multilure" exhibited a very significant dependency relationships to the variables time and catch ($P = -0.969^{**}$ and $P = -0.903^{**}$; $\alpha < 0.01$); the common feature between these two traps is their yellow basal color.

Conclusions: In case of absence of conventional traps, the plastic bottle of 5 liters could, therefore, be used as an alternative and its basal part painted in yellow should increase its efficiency. Due to simple design and less time needed for servicing "Sentrap", increasing its volume and openings and fixing firmly the upper part with a lid screw might improve its efficiency.

Keywords: Traps, catches, *Bactrocera*

PRELIMINARY RESULTS OF AN INTEGRATION PEST MANAGEMENT PROGRAM OF FRUIT FLIES (DIPTERA: TEPHRITIDAE) ATTACKING MANGOES IN CENTRAL TANZANIA.

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Background: There is no proper management program for fruit flies attacking mangoes, despite heavy losses they inflict on the crop, especially after the introduction of *Bactrocera invadens*. There is a need for testing a number of management programs on their efficacies in reducing losses due to fruit flies.

Methods: Trials were conducted in eight selected orchards located in the mango growing areas of Morogoro Region, Central Tanzania. The treatments included (i) orchard sanitation (ii) orchard sanitation, spray of dimethoate 480EC and early harvesting (iii) orchard sanitation, spray of GF 120 containing 0.02% spinosad, male annihilation using methyl eugenol and early harvesting (iv) spray of a locally formulated bait (containing molasses, browsers' waste and crude extracts of *Derris elliptica* as a toxicant) and early harvesting. Each treatment was applied in an individual orchard and replicate twice. Additionally, two orchards of unimproved mango varieties and not subjected to any fruit flies management method were included in the study. Populations of fruit flies in each orchard were monitored using torula yeast placed in McPhail traps. The replication of traps within each site were sub-sampled to construct point wise biased and accelerated bootstrap confidence intervals that were used to compare treatments. Further, fruits were sampled at ripening and individually placed in containers to determine incidences and infestation rates of emerged fruit fly species. Analysis of Variance (ANOVA) was used to compare infestation rates while chi-square was used to compare incidences of fruit flies. Log-linear regression models were used to correlate population of fruit flies, season and weather parameters recorded at each orchard.

Results: Different management programs have shown differences in their efficacies in reducing losses due to fruit flies.

Conclusion: Losses due to fruit flies can be reduced using ecologically based management programs. However, further refinement of some programs is still needed.

Keywords: IPM, fruit flies, mangoes, Tanzania

ADRESS® COUPLED WITH SIT: AN INTEGRATED STRATEGY TO CONTROL MEDITERRANEAN FRUIT FLY.

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Background: Sterile Insect Technique (SIT) has been implemented over Wide Areas all around the world. SIT best results were obtained when *Ceratitis capitata* population was low or very low and this is key point to ensure success of this technique. For this purpose combination of SIT with natural enemies, insecticide sprays or lure and kill technique has been studied in last decades. In this way, we propose to combine chemosterilization technique using Adress® devices with SIT as both affect medfly population reducing fertility. In this case, chemosterilization affects directly wild population and does not kill sterile released individuals and this can suppose a great advantage. Results of combination of both techniques over a wide area compared with only SIT treated area are showed.

Methods: During 2008 and 2009, the efficacy of the combination of two fruit fly control techniques, SIT and Adress®, was tested in three crops: citrus, stone fruit and persimmon. 2000 sterile males were released per ha and week in the whole trial area (50.000 ha, SIT area). Twenty four Adress® devices per ha were hung in 3600 ha (SIT+Adress® area) inside of the whole trial area,. Ten SIT+Adress® plots per each crop and 10 SIT plots per each crop were assessed throughout the trial period. In order to evaluate the efficacy, the male and female population was monitored from August 2008 to November 2009 and injured and the viability of the egg-laying was assessed.

Results: Results showed an important reduction in the *C. capitata* population in plots treated with both techniques versus plots treated only with SIT. A reduction in the percentage of injured fruit in SIT+Adress® plots was observed and the viability of the egg-laying was also minor.

Conclusions: Results show the compatibility of these techniques and provide the possibility of use Adress® coupled SIT to reduce *C. capitata* population in locations with high fruit fly population.

Keywords: *Ceratitis capitata*, chemosterilization, Adress, SIT, sterile

OLFACTORY RESPONSES OF MALE MEDFLIES TO PLANT MATERIAL CONTAINING THE PARAPHEROMONE α -COPAENE.

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Background: The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is a highly invasive species that is considered the most adaptable and polyphagous species of tephritid fruit fly due to its global distribution and its broad range of host plants, primarily tropical and subtropical fruits and vegetables. *Ceratitis capitata* is presently ranked first among economically important fruit fly pests, due to both damage to crops and costs of eradication. Trimedlure is a synthetic chemical that is highly attractive to male medflies and is the standard male-targeted lure used for this species. Medfly response to trimedlure is similar to response to the sesquiterpene α -copaene, a widely-distributed plant compound, and males respond to both host and non-host sources that contain α -copaene. Although α -copaene is reported to be 2 to 5 times more attractive than trimedlure, difficulties in obtaining synthetic α -copaene in sufficient quantities for large-scale trap deployment have prevented its use as a lure. As part of a study on sesquiterpene content of tree cambial tissue, we found that cambial (cambium+bark layers) tissue from avocado, *Persea americana*, contained α -copaene, and that levels were highly variable among different genotypes. Therefore, studies were initiated to determine if these sources of α -copaene were biologically active for male medflies. For comparative purposes, results were compared with responses to cambial tissue from *Litchi chinensis* and *Ficus benjamina*, two substrates known to elicit behavioral responses in male medflies.

Methods: Behavioral bioassays and electroantennography (EAG) were used to evaluate responses of sterile male medflies; and GC-MS analysis was used to quantify the amounts of 13 sesquiterpenes, including α -copaene, common in cambial tissue from four avocado genotypes, from *Litchi* and from *Ficus*.

Results: *Litchi* elicited the highest response and *Ficus* the lowest response, with cambial tissue from the avocado genotypes eliciting intermediate responses that varied significantly among the four types in both the bioassays and EAG experiments. These responses, however, were not correlated with the amount of α -copaene, but were correlated with few other components like α -humulene, which triggered strong antennal responses in EAG.

Discussion: Additional sesquiterpenes may be responsible for the high responses observed with the low α -copaene substrates. Identification of these chemicals may provide a new understanding of the biological basis for the response of male medflies to these wood sources, which could lead to development of new tools for improved detection and control. Attractiveness of α -humulene still has to be tested in bioassays but, this compound triggered a strong antennal response of the male medflies. Although not necessarily attractive, this compound may play a synergistic role to α -copaene for *C. capitata* attraction.

Keywords: *Ceratitis capitata*, bioassays, Electroantennography, olfaction, cambial

USING GIS TECHNOLOGY IN CONJUNCTION WITH FRUIT FLY TRAPPING SURVEY IN THAILAND.

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Background: Geographic Information System, GIS, has been used to support area-wide fruit fly control base on the Sterile Insect Technique, SIT, and related environment-friendly technology in Thailand for 10 years. As a tool, GIS has been used to define project areas, core area and buffer zone, mark trapping site for monitoring fruit fly population dynamic. Difference distance of trapping site were discussed in vision of GIS technician and operation cost.

Methods: Two tephritid species (Diptera: Tephritidae), the Oriental fruit fly *Bactrocera dorsalis* (Hendel) and the guava fruity fly *Bactrocera correcta* (Bezzi) have been considered to be the key insect pests which covered over 160 km² of two areas in Phichit province, one area in Ratchaburi province and one area in Prachuap Khiri Khan province. Steiner traps with methyl eugenol as fruit fly attractant were design as 1 trap per 1, 2, 4, 8 and 10 square kilometers. Traps located were marked by GIS program therefore related distance of radius were 1, 1.414, 2, 2.83 and 3.16 km, respectively. Trapping network weekly deployment frequency was conducted 52 weeks continuously. Fruit fly population index was showed in term of fly/trap/day, FTD.

Results: In four study areas, GIS program generated 158 trap points from trap density in one square kilometers, 80 trap points from trap density in two square kilometers, 36 trap points from trap density in four square kilometers, 18 trap points from trap density in eight square kilometers and 15 trap points from trap density in 10 square kilometers. The result showed that the trap points from trap density in one square kilometers and two square kilometers covered a whole project area but the others did not cover a whole project area. Ranking FTD in seven was classed using ISPM standard in the first three classes and the others classes were classified by the calculation of total data and the expert.

Conclusions: Area-wide Sterile Insect Technique (SIT) programs in a cost-trapping can be a trapping radius of approximately 1.414 kilometers and/or the trap density in two square kilometers.

Keywords: GIS, SIT, fruit fly control, trapping survey, Thailand.

EFFECT OF TRAP DENSITY ON NUMBER OF CAPTURED FRUIT FLIES.

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Background: Fruit fly control has been listed in International Standard of Phytosanitary Measure, ISPM. Surveillance process, data interpretation and assessment are major important systems required in order to thoroughly understand nature of pests and use information as a tool for making decision in conducting activities. Currently, fruit fly control program in Thailand needs much more attention in various aspects than traditional control-based knowledge. Fruit fly investigation is mainly to reduce expense of trap surveillance latest technologies, sterile insect technique, biological control, bait application technique and male annihilation.

Methods: The modified steiner traps using methyl eugenol as an attractant were placed as following: a trap/km², a trap/2 km², a trap/4 km², a trap/8 km² and a trap/10 km², in four fruit orchard areas, Pak-Tau district in Ratchaburi province, Pohpratunchang district in Pichit province, Saklek district in Pichit province and Pranburi district in Prachuabkirikhan province. The total area was 160 km² with 307 traps. Fruit flies were continuously trapped for each seven days/week of 52 weeks. Collected trap data were 31,928. The index to indicate fruit fly population is number of fruit fly/trap/day.

Results: The interaction of the treatments based on fruit fly number and trap density in each area depended on geographical location and fruit fly species. Distribution of *B. dorsalis* at Pranburi district in Prachuabkirikhan province was shown higher than other areas significantly (p<0.05) with 51.56 flies/trap/day. *Bactrocera dorsalis* (2.33 flies/trap/day) from Pohpratunchang district in Pichit province was significantly less than other districts (p<0.05). Number of *Bactrocera dorsalis* were significantly bigger than *Bactrocera correcta* in all area (p<0.05). *Bactrocera correcta* (4.19 flies/trap/day) at Pak-Tau district in Ratchaburi province was significantly higher than other provinces (p<0.05). The areas with high density of *Bactrocera dorsalis*, number of flies/trap/day for 1 trap/km² and 1 trap/4 km² were not significantly different (p>0.05).

Conclusions: In comparison on population density *B. dorsalis* and *B. correcta*, the result showed that *B. dorsalis* was found in higher number in all orchards. The population density of *B. dorsalis* can be the index of fly population. Using the steiner trap with methyl eugenol, a trap/4 km², we can decrease the cost of the survey approximately 75 percent. However we think if this steiner trap would be used in new orchard, the trap density should be a trap/km². After data analysis, the suitable trap density would be made decision later.

Keywords: *Bactrocera dorsalis*, *Bactrocera correcta*, trap density, captured fruit fly, steiner trap

EFFICACY OF KAOLIN AND COPPER TO CONTROL *BACTROCERA OLEAE* (ROSSI) AND SIDE EFFECTS ON NON-TARGET ARTHROPODS.

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Background: Olive fruit fly, *Bactrocera oleae*, is the key pest of the olive crop, demanding annual treatments. Therefore, the development of environmentally friendly control methods is of growing interest. Results of the evaluation in field assays about the efficacy of preventive application of kaolin and copper to control *B. oleae* are hereby reported, as well as the side effects of both treatments on non-target arthropods, paying special attention to natural enemies.

Methods: The field trial was conducted in 2008 in an olive grove at Villarejo de Salvanes, Madrid, using a randomized complete block design with four replicates. Treatments consisted of three applications of kaolin (Surround[®] WP), copper (Bordeaux mixture, Cuprocal[®] WP) and dimethoate bait sprays (Danadim Progres[®] plus protein hydrolysate, Life[®]) as a positive control. *B. oleae* damage was assessed by the number of olive fruits attacked and the developmental stages found. Arthropod fauna of olive tree canopies was sampled by a beating method throughout the growing season. The effect of treatments on abundance and biodiversity (number of species and Shannon index) of non-target arthropods was evaluated. Data were analyzed by means of ANOVA and PRC curves were built to estimate the effect of treatments on natural enemies.

Results: Population levels of *B. oleae* were very high and none of the treatments significantly reduced the percentage of olives attacked. However, a significantly lower number of olive fruits with advanced developmental stages (L3 or pupae) was registered in kaolin treated olive trees at harvest time (20% compared to 64% in the control), what resulted in an important reduction of the damage. No significant changes in abundance and number of species of arthropods were detected. However, the Shannon diversity index was reduced in dimethoate bait plots on the two last sampling dates. PRC analysis showed no significant effect of the three treatments on the natural enemies' community as a whole but a reduction of some specific taxa was revealed. Thus, Phylodromidae, Encyrtidae and Coniopterygidae were negatively affected by the three treatments; kaolin and dimethoate reduced the numbers of the spiders *Tmarus* spp and *Gibbaranea* spp while the populations of the coccinellid beetle *Scymnus mediterraneus* were reduced by Kaolin.

Conclusions: Kaolin seems to be able to reduce the damage of *B. oleae*. However, copper did not control the fly. We detected some slight deleterious effects on non-target arthropods, especially in the case of dimethoate. Longer term trials are needed to better characterize both the efficacy and the side effects of the tested products.

Keywords: Olive fruit fly, kaolin, copper, dimethoate, side-effects

EVALUATION OF MASS TRAPPING EQUIPMENT AGAINST *CERATITIS* SPP. ON THE ISLAND OF LA REUNION.

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Background: Based on several field trials of equipment used for mass trapping of *Ceratitis capitata* conducted in Girona (Spain), similar studies were performed in the island of La Réunion, where two main *Ceratitis* spp. (*C. rosa* and *C. capitata*) coexist. The aim of the study was to evaluate in the field the effectiveness of trapping equipment for *Ceratitis* spp. through comparative studies of trap types, attractants and insecticides.

Methods: All trials were conducted in commercial Citrus orchards and the experimental design used randomized blocks with 4 replicates with a monitoring period lasting from 55 to 60 days. Traps were hung at a height of 1.5 m on Citrus trees. Two rotations per week were made in order to diminish the possible effect of trap location, giving a total of 5 complete rotations. In trials on traps and attractants, the number of males and females were recorded while, in trials on insecticides, alive and dead individuals were taken into account. Data were pooled for each rotation cycle, transformed (arcsine of the square root of the percentages of captures) and analyzed through one-way ANOVA (Enterprise Guide, SAS).

Results: Over all the trials, *C. rosa* was the most abundant *Ceratitis* species captured. Maxitrap® and Tephri-trap traps caught similar numbers of *C. rosa* throughout the trial period whereas Easy-trap® captured significantly fewer adults. Similar results were obtained for *C. capitata*. In the trial on attractants, significantly higher captures of *C. rosa* were found with BioLure Med Fly® (3 dispensers) over the study period, followed by Biolure Unipack® and FERAG® CC D TM. Owing to the lower adults of *C. capitata* recorded (195) no differences were found between the attractants tested. In the insecticide test, there were no significant differences in the numbers of dead *C. rosa* with DDVP and Deltamethrin, which were higher than with Alpha-cipermetrine. A significantly lower number of alive adult *C. rosa* was observed with DDVP, followed by Deltamethrin. Although captures of *C. capitata* were lower, similar numbers of dead flies were observed for all treatments.

Conclusions: The most effective equipment for the capture of *C. rosa*, the species caught in higher percentages in all trials, were Maxitrap and Tephri-trap devices, the attractant BioLure Med Fly and the insecticide Deltamethrin, that could constitute a suitable substitute for DDVP, recently banned in the EU.

Keywords: *Ceratitis capitata*, *Ceratitis rosa*, mass trapping equipment

BAIT STATION VERSUS CHEMICAL CONTROL IN ARGENTINA, PERIOD 2007-2008.

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Background: In this work, we report the results of comparative field studies on chemical control, deployment strategy and efficacy of the bait station methods in the control of the medfly.

Methods: The tests were conducted from November 2007 to June 2008 in San Pedro (Argentina) with the objective of adding new tools in the integrated pest management (IPM) of fruit flies. San Pedro is in the North - East of Buenos Aires Province at 33° 39' latitude South and 59° 41' longitude West at 26.99 m over sea level. Test 1: It was conducted in an orchard of 20 years old (sweet orange var. Valencia late) with density 625 plants/plot (4x4 m). Test 2: In a 6 ha ten-year-old organic orchard (sweet orange var. Washington Navel), with trees spaced (3x4 m) separately (density 825 plants/plot). On both of them, the following four treatments were tested (each in a one ha plot within the orchard): a) Mass trapping: One BBS (Gum stick (classic) 9% AA, 5% TMA, 1% Spinosad, supplied by R. Heath, USDA/ARS, Miami, FL)) placed in every second tree; b) Bait stations: 100 BBS distributed in four rows (25 per row) of the one ha plot; c) Ground bait sprays (chemical control locally in use) using Spinosad formulated bait (Flipper) containing 0.02% Spinosad; and d) Untreated control (no control). Medfly population densities in each plot were assessed every week by placing five Multilure baited trap (Better World) with three components food-based synthetic lure containing Ammonium Acetate, Putrescine and Trimethylamine (BioLure, Suterra LLC) and water plus Triton added to the base in order to retain attracted flies. The traps were sampled after 24 h and the number of flies captured was determined. The fruit was sampled once a week in all treatments in order to assess its infestation levels. One hundred fruits were sampled in each treatment and were dissected. The larvae found were put in Petri dish until adult emergence. The fruit infestation levels in each treatment are given.

Results: In both tests the Mass Trapping Treatment (a) showed similar control and protection to the Ground Bait Treatment (Spinosad) (c) in fruit infestation levels and cached adults in Multilure traps. The Bait Station Treatment showed a good control in Test 1 (very closed to Ground Bait and Mass Trapping results) but in Test 2 its performance appeared to be not as good probably due to the exceptionally high populations of Medfly. Nevertheless, even under these unfavorable conditions the Bait Stations Treatment provided a remarkable control when compared with the untreated control.

Conclusions: We consider that the three treatments protected the orange orchards during the entire time of fruit susceptibility. The Mass Trapping and Bait Station methods provide a useful medfly management tool which can be combined with other IPM methods used in the area. These procedures have the advantage of being environmentally friendly, not polluting the atmosphere and preserving the beneficial entomofauna. This technology is very important in integrated or organic fruit production, and for several markets in which the control of insecticide residue is important.

Keywords: Medfly, bait station, alternative control, mass trapping, Fruit Fly SIT.

ENRICHED GINGER OIL, A POWERFUL SEXUAL ATTRACTANT FOR TRAPPING THE MALES OF SOME *CERATITIS* SPP.

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Background: Since the 60's, trimedlure has been the main para-pheromone used to attract the males of a series of *Ceratitidis* spp. for monitoring or detection purposes. In recent years, within a FAO-IAEA coordinated programme, in the framework of SIT (Sterile Insect Technique) programmes, the strong attractiveness of Ginger Root Oil (GRO) for the males of some *Ceratitidis* spp. has been confirmed. Based on these results, it appeared useful to compare the attractiveness of a recently available commercial dispenser of Enriched Ginger Oil (Insect Science SA) with that of a standard trimedlure dispenser, in field trapping experiments. More recently, field-cage tests were carried out to determine the factors (age, food status, mating...) influencing the male response in both species.

Method: Four field experiments were conducted in 2009-2010 on different crops. The first one was carried out in a Citrus orchard situated in Bassin Martin, in the south of the island, at an altitude of 290 m. The second trial was carried out in another Citrus orchard, in Petite Ile in the south (alt.: 300m) and the third one in Etang-Salé, in the south-west, in a chilli plot (alt.: 10m). Finally a fourth trial, set up in Piton Armand on the East in a Chinese guava plot (alt.: 450m), is still in progress. For each trial, two types of attractants were compared in Tephri-traps (Sorygar, Spain): Enriched Ginger Oil (EGO) ("Pherolure", Insect Science SA, Tzaneen, South Africa) and trimedlure (Agrisense BCS Ltd, Pontypridd, UK). The attractant dispensers were placed in a small plastic basket at the top of the trap, while a strip of DDVP was placed at the bottom of the Tephri-trap to kill the adult flies. From four to six replicates were carried out depending on the trial. Traps were monitored weekly for recording the number of caught males, then rotated. For each species, results were analysed with a GLM (Poisson family, logit link) on the number of flies caught per trap, using the soft ware R.2.8.1.

Result: In trials 1, 2 and 4, the populations of *C. capitata* were lower than those of *C. rosa*, while in trial 3, in the dry south-west of the island, the medfly dominated. In all trials (except trial 4, still in progress), for both species, EGO attracted highly significantly more males than did trimedlure (glm, 1 d.f., $p < 0.001$). Indeed, depending on the trial, EGO attracted between 2.7 and 4 times more males of *C. capitata* and between 6.1 and 16 times more males of *C. rosa*. In all trials, the factors 'date' and 'replicate' also significantly influenced the number of caught males.

Conclusions: In the different field trials, the EGO dispenser proved much more attractive for the males of *Ceratitidis* spp. than the trimedlure dispenser. This difference was particularly striking in the case of *C. rosa*. The strong para-pheromonal attractiveness of this essential oil, might be linked with the presence of α -copaene. Because of its strong attractiveness for the males of these two *Ceratitidis* spp., EGO could be strongly recommended for use in detection programmes, and even tested for Male Annihilation experiments. It would also be interesting to evaluate its attractiveness for the males of other African *Ceratitidis* spp.

DESIGNING CONTROLLED RELEASE DEVICES: FORMULATING FRUIT FLY ATTRACTANTS AND COMMERCIALY AVAILABLE INSECTICIDES.

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Background: The development of controlled release devices (CRDs) can be summarized as being dictated by the following factors: (1) the chemistry of the semiochemical (2) the targeted level of attractancy (3) economics. The design of the CRD will account for the volatility and solubility of the attractant, for example equal quantities of methyl eugenol and cue lure will not have the same longevity. To obtain equal longevities, formulations for methyl eugenol have a higher loading, and a different ratio of vinyl polymer to compensate for the increased volatility of the molecule. Furthermore, formulations for cue lure are developed to not last beyond the required field-life, thus preventing residual attractant from remaining within the CRD. In summary, semiochemicals are formulated to achieve optimal release from polymer-based CRDs. A general practice of monitoring fruit flies in the United States, specifically regions such as California and Florida, entails absorbing an attractant and an insecticide onto a cotton wick and positioning the combination into a Jackson Trap with a sticky bottom. The preparation of the cotton wick is laborious and the users are exposed to liquid insecticides such as Naled (Dimethyl 1,2-dibromo-2,2-dichloroethyl phosphate) and DDVP (Dimethyl 2,2-dichlorovinyl phosphate). Combining the attractant and the insecticide within a polymeric CRD would minimize exposure to insecticides and eliminate the added task of baiting wicks.

Methods: A study was conducted on native populations of *Bactrocera dorsalis*, Oriental Fruit Fly, on the Big Island of Hawai'i. The 8-week trial compared baited wicks and the Scentry Methyl Eugenol Cone combined with the following commercially available insecticides: Naled, DDVP, Spinosad, and Rynaxypyr. The lures and wicks were baited with 6 ml of attractant and 5% of the insecticide.

Results: The DuPont Rynaxypyr formulation demonstrated strong competitive insecticide activity to the organophosphate formulations at a smaller percentage or loading of insecticide per device.

Conclusion: Every one of the all-in-one products present in the study were competitive to the loaded wicks, offering field technicians a viable alternative to the old method of baiting cotton wicks.

Keywords: *Bactrocera*, Methyl Eugenol, Cue Lure, Rynaxypyr.

COMPARISON OF EFFICACY OF BACTROGEL AND SUCCESS APPAT FOR THE CONTROL OF CUCURBIT FRUIT FLY, *DACUS DEMMEREZI*, IN MADAGASCAR.

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Background: Cucurbit production is one of producer's income sources. Unfortunately, this culture either cultivated or wild is submitted to *Dacus demmerezi*, the cucurbits fruit flies attacks. The harvest lost could be assessing at 85%. In order to implement a survey strategy against this pest the team thinks that it is judicious to find an effective product which would be advised to the producers

Methods: All experiences have been lead on the ACM Society parcel. Maize has been growth at the four parcels corner and Mac Phail trap with cue lure has been placed in the center. Every fifteen days, captured flies in the trap has been counted and maize has received application with either Bactrogel or Success Appat

Results: The graph of fruit flies population evaluation has shown that *Dacus* has a good response as well to Bactrogel as to success appat but there is little bit difference on capture.

Conclusion: We conclude that Success Appat is more effective than Bactrogel

Keywords: Success Appat, *Dacus*, Bactrogel

MASS TRAPPING OF *CERATITIS CAPITATA* AND NON-TARGET CONTROL FLIES.

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Background: Control of fruit fly *Ceratitits capitata* (Diptera: Tephritidae), in Israeli apple orchards is based on airborne, wide-area, ULV spraying with malathion mixed with protein hydrolysate (PH). In IPM orchards, where use of malathion and other organophosphates is not advisable, attractant-formulation devices are used, without pesticides (Cera Trap, Olike) or with a selective pesticide (spinosad) (Biofeed, Fructect06). This study examined attraction of flies, including *C. capitata* (male and female) and non-target control flies to such devices and systems.

Methods: The first trial, in four commercial apple orchards (each about 2 ha) in northern Israel, used four different attractant devices to control *C. capitata*: 1) Cera Trap at 100/ha; 2) Olike, 100/ha; 3) Biofeed, 150/ha; 4) Fructect06, 150/ha. The first two are non-toxic devices based on attractant formulations of PH and Torula yeast, respectively. Attraction to the last two is based on an odorous mixture of pesticide (PH and spinosad), in combination with color and form (a square yellow board with a red circle). Six such devices were placed randomly in each treatment orchard after some modifications to facilitate fly trapping: 1) The Cera Trap and Olike bottle were cut off below the fly entry hole. To avoid drowning the flies the piece of net and the DDVP plug (the lethal component) were placed above the attractant formulation; 2) The Biofeed and Fructect06 were covered with transparent covers and sprayed with insect glue. The devices were checked every week from July through December. The second trial involved airborne wide area control in citrus orchards; the same attractant formulations were tested, all of them poured from bottles. The trial was arranged in five random blocks with 25 m between devices. The devices were checked weekly from January through April. To prevent possible influence of location of fly trapping (i.e., fly hot spot) the device was moved to the adjacent location within the same block after being checked.

Results: Significantly more trapping of *C. capitata* in apple orchards (Jul.-Dec.) were found with Olike (average of 25.7 per device) and Cera Trap (23.0) than with Fructect06 (5.5) and Biofeed (0.4); the male:female ratios were 1:4, 1:2, 1:1.7, and 1:1, respectively. All devices also trapped non-target flies (NTF) at average rates of 290.3, 310.0, 56.2 and 51.2 per device, respectively. The trapping ratios of *C. capitata*:NTF were: 1:11.3, 1:13.5, 1:10.2, 1:128.0, respectively. In citrus (Jan.-Apr.) *C. capitata* trappings averaged 1.4, 12.4, 2.8 and 9.4 per device in Olike, Cera Trap, Fructct06 and Biofeed, respectively, with male:female trapping ratios of 1:6, 1:2.3, 1:1.8, 1:1.6, respectively. The average rates of NTF trapping were 488.8, 898.0, 621.8 and 168.8 per device, respectively, and the *C. capitata*:NTF trapping ratios were: 1:349.1, 1:72.4, 1:222.1, and 1:17.9, respectively.

Conclusions: The results clearly show the problem presented by the higher rate of NTF trapping, with regard to an IPM strategy for controlling *C. capitata* if attractant devices using selective products are to be considered. The high rate of NTF and low rate of *C. capitata* trapping must be addressed in development of specific attractant formulations.

Keywords: Mass trapping, *Ceratitits capitata*, Non-target flies.

TRANSGENIC ORANGE FRUIT WITH REDUCED LIMONENE CONTENT IN THE PEEL IS LESS ATTRACTANT TO *CERATITIS CAPITATA* (WIEDEMANN).

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Background: *Ceratitis capitata* (medfly) males form loose aggregations termed leks, performing sexual signaling by emitting a sexual pheromone. Receptive females visit leks and choose their mates on the basis of their physical characteristics and their courtship performance. The secondary compounds produced by plants, many of them are volatiles, have signaling functions. In *Citrus*, the peel is embedded of thousands of oil glands that contain terpene volatile compounds, being limonene the most abundant one (up to 97% in orange fruits). The high amount of limonene that accumulates in orange oil glands suggests an important biological role for this terpene compound in fruit odour interactions with the biotic environment. However, some studies have suggested mating advantages when adult males were exposed to different volatiles derived from orange fruits.

Methods: Transgenic plants expressing the complete cDNA of a citrus limonene synthase gene (*CiTMTSE1*) in antisense orientation (AS) were used in this study. Pure synthetic terpene compounds were used in laboratory experiments. Neonate larvae and 5 day-old laboratory mass-reared flies were used in laboratory, greenhouse and wind tunnel bioassays.

Results: Transgenic plants had a decrease of two orders in magnitude of limonene accumulation in fruit peel. Consequently, the accumulation of other monoterpenes and sesquiterpenes decreased while that of monoterpene alcohols increased. Wind tunnel bioassays with medfly males exposed to pure compounds revealed a clear attraction to linalool and limonene volatiles. In no-choice experiments, AS and control fruits were damaged at comparable levels by medfly females. Nevertheless, wind tunnel bioassays with AS and control fruits showed that males were not only not repelled but significantly more attracted to control fruits (more than ten times; 32% vs. 2%), indicating that limonene (and likely other terpene) emission were actually insect- attractants to the fruit.

Conclusions: These results provide a much more comprehensive view of the role of terpene volatiles as attractors of medfly in *Citrus* fruits.

Keywords: Limonene, volatiles, antisense downregulation, *Ceratitis capitata* behaviour

TRANSPARENT VERSUS YELLOW COLOR IN TRAPS AGAINST *BACTROCERA OLEAE* (ROSSI).

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Background : Currently, yellow is the most frequent color in traps against Tephritidae family. The first Macphail traps that began it way made with transparent glasses, today, because the manufacturing, transport and prices, have derived into plastics and yellow color as the reference. The fundament of Easy trap[®] (Sorygar,Co) (as the main different with Mcphail) is to have two opposite “Ventury” holes that makes the vacuum inside the trap when the air is coming in, forcing out the smell of the bait when the air is coming out. Easy trap has a high efficiency degree when capturing fruit flies. It is working with solid bait (Biolure[®] Suterra,Co) as well as liquid baits (Hydrolysate protein) but even though to be airtight the farmers don’t close it well and the liquid spill. A new version “Easy Blister Trap[®]” still keeping the Easy trap concept (ventury holes) but it is a completely transparent and completely sealed without any manual interaction. We have carried out an experiment to determine the variability of both traps capturing *Bactrocera oleae* and studying if it have differences on the attracted parasitoids and predators.

Methods: A ecological olive farm of 4 ha. sited in Villarejo (Madrid Province) was chosen to hung the traps, Easy (yellow) and Easy Blister Trap (Transparent) both baited with Tephri Lure[®] 12% from 10 June to 12 November of 2009 (165 days). The statistical design was 4 replicates of both traps on alternate olives and consecutive rows separated each other (12x24 m). The check was made each 15 days, counting males and females as well as other insects. A statistical analysis of variance was carried out with the data.

Results: No significant differences ($\alpha = 0.05$) between the captures of both traps (males and females) although in all checks the transparent trap capture more flies tan the yellow one. Regarding numbers of non-target arthropods captured, the percentages of natural enemies were 6,1% for Easy trap and 5,6 for Easy Blister Trap but no statistical significant differences were observed between these numbers, either in the case of predators or parasitoids.

Coclusions: According with the results; neither *Bactrocera oleae*, nor predators, nor parasitoids change their behavior when trap color is changed from yellow to transparent. This experiment is giving some evidences that the most important factors for trap designing is the kind of attractant and the way the trap spread it in the air and not the color of the trap

Keywords: *Bactrocera oleae*, easy trap, easy blister trap, natural enemies

AMMONIUM PHOSPHATE VERSUS HYDROLYSATE PROTEIN: WHICH ONE TO BE USED IN MASS TRAPPING AGAINST THE OLIVE FLY *BACTROCERA OLEAE*, (ROSSI).

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Background: Mass trapping is one of the methods to control olive fly when insecticide is forbidden, case of organic grows. Olipe trap baited with Ammonium Phosphate is a well-known trap extensively used in many countries. Many years ago we have demonstrate that hydrolysate protein is a better attractant than Ammonium Phosphate against olive fly (IAEA programmes). This method arise a high cost of money and hand work if we not guarantee the maximum of captures that makes a free damage harvest. The authors are working several years with a new protein hydrolysate (Tephri Lure[®], Sorygar,Co) needing to check that there are no errors. In this poster we present the results of four simultaneous trials carried out in four separated sites with very different climates.

Methods: The experiment was carried out in four localities of Spain: Jaén (the greatest area of olive culture), Almería, near the Mediterranean sea, and Madrid (2), middle of Spain with continental climate. We have used two kind of traps: Olipe (water mineral bottle) with 4 holes (1,5 cm ϕ) and Easy Blister trap a new version of “Easy Trap[®]” Sorygar,Co) that keeping the Easy trap concept (ventury holes) but it is a completely transparent and completely sealed without any manual interaction. Each trap was baited with Ammonium Phosphate 4% and Tephri Lure 12% (from origin with borax and glycol). The traps were hung on the interior of the olive tree. Four replicates were placed randomly in a square surface of sixteen olive trees. (12X12m.). The check of traps was monthly, males and females were counted just like the different arthropods. The statistical analysis of variance was made with the change of variable $x'=(x+1)^{1/2}$

Results: In all localities were the experiment was carried out the hydrolysate protein has captured much more flies independent of the type of trap. Easy Blister Trap was better than Olipe Trap when both trap are baiting with Ammonium Phosphate, but they are not significant differences when they are baiting with hydrolysatate protein. (F=11,3; p<0,0001). The total amount of captures were: Easy Blister/Phos 5535 flies, Olipe/phos 2427 flies; Easy Blister/hydrolysatate 16417 flies and Olipe/hydrolysatate 16417 flies.

Conclusions: The hydrolysatate protein is much better attractant than Ammonium Phosphate independent of the type of trap. It is necessary a economical study to see if capturing the high number of flies as have showed the protein, the cost of the Mass Trapping Method is compatible with a ecological free damage harvest.

Keywords: *Bactrocera oleae*, olive fruit fly, mass-trapping, amonium phosphate, tephirilure

EFFECT OF SUBLETHAL CONCENTRATIONS OF NEEM EXTRACTS ON THE REPRODUCTION AND LONGEVITY OF THE MEDFLY, *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE).

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Background: Fruit flies show high vitellogenesis at the adult stage, which represents a good moment to verify the physiological effects of neem extracts. The sublethal concentrations (LC₁₅, LC₃₀ and LC₄₅) of aqueous extract from seed kernels of *Azadirachta indica* A. Juss for *Ceratitidis capitata* (Wied.) adults were estimated applying bait (extract + honey) on 'Cravo' lemon seedlings. Subsequently, the effect of different extracts in these sublethal concentrations (39, 225 e 888 ppm, respectively) on the longevity, fecundity and the fertility on the 1st, 4th, 8th and the 12th days of oviposition was evaluated.

Methods: As first step, using the aqueous extract from neem seed kernels, the LC₁₅, LC₃₀ and LC₄₅ were estimated for adults exposed to a bait (a solution of 20 % honey + neem) in cages containing a citrus seedling. After that, those concentrations were used for a second bioassay. It was used plastic cages where it was fixed a cotton wick which was in contact with a plastic vial with 12.5 ml of the extracts (seed kernel aqueous extract, leaf extract in methanol and branch extract in dichloromethane). Water or 5 % acetone were used as controls. Two couples were released in each cage and an artificial diet (hydrolyzed yeast + sugar) was offered *ad libitum*. The sublethal concentrations were estimated by probit regression analyses using the SAS 9.1 software. The experiment was completely randomized with four replicates per treatment and was conducted under environmental controlled conditions (25 ± 2°C, 60 ± 10 % RH and 14 h photophase).

Results: At these sublethal concentrations, the longevity of males and females and the pre-oviposition period were not significantly affected. The branches extract in dichloromethane at the highest concentration (888 ppm) affected significantly the fecundity and the fertility at the 8th day, reducing in almost 80 % the number of eggs laid and in 30 % the egg hatching.

Conclusions: The estimated LC₁₅, LC₃₀ and LC₄₅ were 38.68, 225.19 and 887.86 ppm for the aqueous extract of neem seed kernels in the bait. The branches extract in dichloromethane at the concentration of 888 ppm affected significantly the insect fecundity and fertility.

Keywords: Medfly, *Azadirachta indica*, sublethal concentrations, reproduction.

EFFECT OF SPINOSAD, KAOLIN AND PROTEIN BAIT SPRAY ON *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE) POPULATION IN CITRUS ORCHARDS.

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Background: Phytosanitary practices based on the extensive use of insecticides in citrus groves to control the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) may cause the resurgence of resistant strains of this pest, the emergence of secondary pests by elimination of their natural enemies, and the growing public concerns over issues related to public health, environmental quality, and food safety. Therefore, there is an urgent need to develop an alternative and environment friendly method for *C. capitata* management. The purpose of this study was to demonstrate the efficiency of the treatment with the kaolin, spinosad and protein bait spray (BAT) and how these different treatments may be associated for a better control of *C. capitata* in several citrus varieties.

Methods: Citrus plots of Clementine (*Citrus reticulata*, var. Cadoux), sweet orange (*Citrus sinensis*, var. Hamlin), sweet orange (*Citrus sinensis*, var. WashingtonNavel), and Clementine Mandarin (*Citrus reticulata*, var. Marisol) were treated with kaolin for 7 days at two doses.

Results & Conclusions: The plots treated with the kaolin continuously for seven days showed a lower number of *C. capitata* captures and fruit infestation compared with those treated with BAT or spinosad. Kaolin used at a low dose (1.4%) showed a similar level of infestation as spinosad. However, at a high dose (3%), a small reduction in fruit infestation is obtained compared to spinosad. Addition of the hydrolyzed protein does not seem to enhance spinosad effectiveness. The feasibility of combining resistant citrus varieties with these alternative methods is discussed and would be considered in the development of an integrated pest management strategy against medfly in citrus groves.

Keywords: Citrus, *Ceratitis capitata*, Kaolin, Spinosad, Pest management.

MONITORING *RHAGOLETIS ALTERNATA* FALLEN – A SERIOUS PEST OF ROSE HIPS.

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Background: Due to their biochemical compounds the interest of growing rosehips, the fruits from different *Rosa* species (mainly section *Caninae*), has increased the last years. In orchards growing raw material to health promoting products there is a demand for organic production. *Rhagoletis alternata* is the most serious pest in wild-growing as well in cultivated rosehips. From a previous study, a high percentage of the wild-growing plants had infested fruits. Initial study of phenology of *R. alternata* has been performed.

Methods: *Rhagoletis alternata* was studied during three seasons in an orchard in the south of Sweden. The plant material originated from a plant breeding program and included several *Rosa* species. Using three-dimensional unbaited yellow sticky traps (Rebell amarillo) adult flies were monitored from June to September. The weather data was collected and the degree-days have been calculated.

Results: In 2007 more flies were captured on traps compared to the same period in 2008 and 2009. In all three years the peak of fly capture occurred at the same time on calendar basis. Differences in captures between traps showed possible genetic variation in *Rosa* species in their chemical content.

Conclusions: The study demonstrated that the Rebell amarillo was effective for capturing *R. alternata* flies. Further study will probably show interaction between the pest and different *Rosa* genotypes.

Keywords: Rosehip fly, traps.

USE OF FOLIAGE BAIT SPRAYS IN THE REDUCTION OF *BACTROCERA INVADENS* (DREW, TSURATA AND WHITE) DAMAGE ON SWEET ORANGE VARIETIES.

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Background: Damages caused by fruit flies have been identified as major limiting factors to citrus production in Nigeria. Recently, a newly introduced species *Bactrocera invadens* caused economic yield losses in many producing areas. The majority of sweet orange producers in Nigeria are small-holders who do not often adopt standard citrus production practices, usually due to lack of the necessary funds. The present study therefore seeks to evaluate the application of locally-made protein bait sprays in different sweet orange varieties in the reduction of fruit fly damage.

Methods: In 2006 and 2007, one, two and three spot applications of locally-made protein bait mixed with chlorpyrifos insecticide were made separately to the foliage of Agege, Valencia Late and Parson Brown sweet oranges arranged in randomized complete block and replicated thrice at the National Horticultural Research Institute, Ibadan. Sweet orange stands with no treatments were maintained as control. The candidate trees were picked at random. Efficacy of the bait sprays in reducing fruit attack was assessed by randomly sampling 10 ripe or ripening fruits per stand. Each spot spray covered 50cm x 50cm of the foliage. Sweet orange stands with no treatments were used as controls. Efficacy of the bait sprays in reducing fruit attack was assessed by close observation of fruit fly entry spots (with the aid of hand lens) in 10 ripe or ripening fruits randomly sampled per stand. The number of fruits that dropped underneath each candidate tree due to fruit fly damage was recorded. The fruits were picked and buried away from the site. Confirmation of fruit fly species causing sweet orange damage was achieved by rearing fruit flies in attacked fruits placed inside wooden cages till adult emergence. Data were subjected to Analysis of Variance (ANOVA) procedures and means of significant tests were separated using Student-Newman-Keuls test at $P \leq 0.05$.

Results: The bait sprays significantly ($P < 0.05$) affected the number of damaged fruits in 2006 and 2007. The control stands had significantly higher number of attacked and dropped fruits (Maximum 8 and 25 respectively) than all the treated stands (maximum of 2 and 13 fruits respectively). The least number of attacked fruits was observed in stands given three spot applications but was only significantly lower than one-spot application. Agege had significantly ($P < 0.05$) higher number of dropped fruit than Parson Brown. Although Valencia Late had the least number, it was not significantly different from that of Parson Brown.

Conclusion: The study shows that three spot applications were most efficacious in reducing fruit damage, while Valencia Late variety was least damaged. The method can be adopted by low income farmers because it is cheap to produce.

Keywords: citrus, fruit fly, fruit drop, Nigeria, yeast bait spray

IMPACT OF WEAVER ANTS (HYMENOPTERA: FORMICIDAE) ON MANGO FRUIT FLY (DIPTERA: TEPHRITIDAE) DAMAGES IN BENIN IN 2008-2009.

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Background: In sub Saharan Africa the mango fruit industry is facing with the problem of fruit flies. Among the numerous species that attack the mango, a new invasive species, *Bactrocera invadens*, is causing especially extensive damage compared to the native fly species during the rainy season. It has a number of comparative advantages such as good flying skills and competitive demographic parameters which allow it to re-infest orchards relatively quickly. Making more efficient use of natural enemies can be of great benefit to the growers. As Asian growers are using Asian weaver ant (*Oecophylla smaragdina*) since many centuries it made sense to test the ability of African weaver ant (*Oecophylla longinoda*) to be an effective biological control agent in Africa. We have studied the ant impact on early cultivar (cv) in 2006-2007. It was interesting to do the same kind of experiment on season and late cv.

Methods: In Benin, we tested the impact of weaver ants on both *B. invadens* and *Ceratitis cosyra* on 5 mango cv (Ifac 3, Améliorée du Cameroun, Kent, Smith and Brooks). The first three ones are season cv and the last two ones are late cv. We used three blocks in 2008 (Ifac 3, Kent, Smith) and three other blocks in 2009 (Améliorée, Smith and Brooks) with two treatments each (i) without ants = control, (ii) with ants. Each treatment was repeated 10 times. Data were analysed by one-way ANOVA followed by Student Newman Keuls test. Analysis was done using the SAS Statistical Program(SAS 2003).

Results: Mean damage inflicted by fruit flies was significantly reduced by 58% in 2008 on Ifac 3, Kent and Smith between mango trees with ants vs control trees. In 2009, mean damage inflicted by fruit flies was significantly reduced by 81% in other blocks of Améliorée du Cameroun, Smith and Brooks. The control mangoes cv Kent, Smith and Brooks had the highest infestation with *B. invadens* populations. It is normal because a consistent population increase of *B. invadens* in the early rainy season caused considerable damage to mid season (Kent) and late cultivars (Smith, Brooks).

Conclusions: The use of *Oecophylla* colonies is well-suited for perennial cropping systems in sub Saharan Africa, because they are efficient, constantly available, widespread and self-regenerating. Practical information about the use of weaver ants in fruit fly control should be made available to all those involved in the mango fruit industry at every level.

Keywords: conservation biological control, *Mangifera indica*, fruit flies, West Africa.

THE STUDY ON THE APPLICATION METHODS OF PROTEIN BAIT TO ORENTIAL FRUIT FLY.

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Background: Oriental fruit fly, *Bactrocera dorsalis* (Hendel), is a tephritid fruit fly that is a serious pest of a wide range of tropical fruits. Application protein bait was one important measure to prevention and control *B. dorsalis* (Hendel). Using trap bottle and spot spray were two important means of application protein bait.

Methods: The effect some factors to attractant effectiveness were studied in this paper, i.e using methods, amount and cycle of protein bait, and comparing the application effect with different host orchards, different protein bait and different locations.

Results: The effect of diluting two-fold was best regardless of means, and the number of attractant and death were 5.33 and 6.67 respectively. The optimal use amount of two methods were 15mL and 30mL, even though the attractant effectiveness increased with the increase of using amount. The life cycle of two means were the same as 5 days, and the the number of attractant and death were 12.33 and 13.33 on the first two days. The results of using trap bottle founded that the attractant effectiveness can be increased by raising pH value properly, and the best value of pH was 6. The attractant effectiveness in star fruit orchard was better than oranges and grapefruit orchards, and the attractant number was 5.00. There was no significant difference between home-made protein bait and Prima, the attractant number were 7.33 and 9.33 respectively, which significantly higher than GF-120. The attractant number of pake, orchard and campuses were 11.73, 7.00, and 5.33 respectively. The results of spot spray showed that the effect was best when adding 0.20% amount of malathion, and the adults number of deaths was 7.67.

Conclusions: The methods have some benefits of guiding on field management.

Keywords: protein bait; *Bactrocera dorsalis* (Hendel); application method; trap bottle; spot spray

Workshop
Longevity, Ageing & SIT

TRANSCRIPTIONAL CHANGES RELATED TO MATURATION AND MATING IN MALE AND FEMALE *CERATITIS CAPITATA* WIEDEMANN.

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Background: The medfly, *Ceratitidis capitata*, is a highly invasive agricultural pest that has become a model insect for the development of biological control strategies. These control strategies are reliant on knowledge of the behaviour, physiology and molecular genetics of medfly reproduction. The recent availability of expressed sequence tags derived from adult medfly heads and embryos has permitted the development of microarrays for high throughput gene expression profiling. The genes that show changes in expression as a result of maturation and mating could potentially be used as targets for the development of novel medfly control strategies.

Methods: A high density oligonucleotide microarray was designed based on 11885 assembled expressed sequence tags from medfly cDNA libraries derived from 0 - 8 day old male and female heads and from 0 - 36 h embryos. Complementary RNA derived from virgin immature, mature and mated males and females of the ISPRA strain were labelled and hybridised to the microarrays using standard Agilent protocols. The microarray data were filtered, normalized and analysed using TIGR MIDAS, MeV and GEPAS software. Gene Ontology and GO Term Enrichment analysis analyses were performed using GO Slimmer and GOEAST, respectively. The microarray-derived gene expression results were confirmed by comparison with the expression patterns of 12 genes obtained using real-time quantitative PCR (qRT-PCR). Three reference genes were used for relative quantification normalization.

Results: A microarray based gene expression approach was used to compare the head transcriptomes of adult male and female medfly at differential physiological stages, sexually immature and mature virgin individuals and mated individuals. Particular attention was focused on the expression patterns of transcripts belonging to reproduction, behaviour, sensory perception of chemical stimulus, and immune system processes. Compared to the broad transcriptional changes during the maturation of the female, post-mating changes in females were modest, suggesting that in the medfly mating does not trigger extensive transcriptional changes in the head. Of particular interest is the apparent lack of mating-induced immune responses in the medfly female, compared to *D. melanogaster*, which may be the result of the different reproductive strategies of these species.

Conclusions: Our study has revealed intriguing transcriptional changes during medfly maturation and mating. This study will encourage further investigations on numerous questions regarding the biology of this and related species. Apart from increasing our understanding of the molecular machinery behind these biological processes, the implicated genes may represent important targets that could be used to control populations of this pest species.

Keywords: *Ceratitidis capitata*, microarray, immune response, reproduction, gene expression

GENOME-WIDE TRANSCRIPT PROFILES TO UNVEIL *CERATITIS CAPITATA* (WIEDEMANN) CANDIDATE GENES INVOLVED IN AGEING.

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Background: The success of a SIT program depends greatly upon releasing males at optimal sexual maturity and that they live long enough to successful mating with wild females. Consequently, information the knowledge of wild female reproductive status (mature or not) at the time of the sterile male release is a factor to be considered for the success of the SIT. Ageing is one of the most complex biological processes being determined by both, genetic and environmental factors. In *C. capitata* sex-specific life span patterns have been described as resulting from an underlying “constitutional” longevity minus the deleterious effects of reproductive biology, and sex-related behaviour. The role of genes in determining the rate of ageing and the primary mechanism underlying the ageing process are the subject of intense investigation. In recent years, sequencing analysis has become an important tool in molecular biology.

Methods: In this work we present a genome-wide expression analysis based on Suppressive Subtractive Hybridization technique (SSH) and Expressed Sequence Tag (EST) sequencing and macroarray expression analysis to identify signature genes related to the ageing-maturing process in *C. capitata*. Three differentiated reproductive (immature, mature and old adults) times have been used to perform SSH in circular scheme, both to females and males. After RNA extraction and SSH, all the subtracted cDNA were cloned and single-pass sequenced. After comparisons to Genbank, consensus sequences were printed in a membrane and expression was measured by macroarray hybridization in each select time and compared in between by using GEPAS software.

Results: We have sorted out 94 unigenes from 873 single-pass ESTs, of which 57% have homology with known genes. Ageing-maturing process in *C. capitata* presents a marked expression pattern accompanied by the increase of transcription level of genes involved in reproduction. In females, the maximum peak of expression (for vitellogenins and chorion proteins) is obtained at 5 days-old, whereas in males it is reached at the age of 15 days (for the male specific serum proteins). Other identified cDNAs with a differential expression pattern would be also candidates but deserves further studies, as they belong to the 43% unknown function class.

Conclusions: These genes and the new candidates would allow proposing a transcriptional profiling protocol to determine the reproductive status and putative age of medflies in field.

Keywords: Medfly, ageing, SSH, ESTs sequencing

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