MEMORIAS

PROCEEDINGS

7a Reunión del Grupo de Trabajo en Moscas de la Fruta del Hemisferio Occidental

7th Meeting of the Working Group on Fruit Flies of the Western Hemisphere

Noviembre/November 2-7, Mazatlán, Sinaloa, México

Memorias

de la

7ª Reunión del Grupo de Trabajo en Moscas de la Fruta del Hemisferio Occidental

Proceedings

of the

7th Meeting of the Working Group on Fruit Flies of the Western Hemisphere

Mazatlán Sinaloa, México

Noviembre/November 2 – 7, 2008

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In addition to the organizing committee, many other people helped to make the 7th Meeting of the Working Group on Fruit Flies of the Western Hemisphere a success, contributing with high enthusiastic in each task that were required (e.g., at the registration desk, at transport of supplies and equipment, support at poster set up, etc.) In particular, thanks to those members who suggested topics for the scientific program, and/or agree to serve as session moderators. Finally, we thank to the staff of different institutions (SAGARPA, AIEA, USDA, CESAVESIN, IICA), by their support and confidence in the organization of this event.

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Conferencia Inaugural/Inaugural Conference

Importancia de la Horticultura Mexicana y su Componente Fitosanitario: El Caso de las Moscas de la Fruta

Javier Trujillo Arriaga

Director General de Sanidad Vegetal. SENASICA-SAGARPA. Av. Guillermo Pérez Valenzuela No. 127 Colonia del Carmen Coyoacán, C. P. 04100 México, D. F.

El trabajo realizado en el control oficial de plagas agrícolas en México, a lo largo de más de 100 años, se ha transformado y adecuado a los tiempos y exigencias de cada etapa de la vida nacional, de acuerdo a lo establecido en las legislaciones fitosanitarias de 1924, 1940, 1970, 1994 y las recientes modificaciones durante 2007 a la Ley Federal de Sanidad Vegetal. Esta Ley mandata a la autoridad federal la función de regulación oficial, para salvaguardar la sanidad vegetal de México, bien público que ninguna otra instancia diferente a la federal, podría resguardar.

Uno de los pilares dentro de la economía nacional es la producción agrícola, por ejemplo cada año se cultivan alrededor de 1.7 millones de ha de hortalizas y frutales; donde se producen 12 millones de ton con un valor comercial de 4,500 millones de dólares, por tal motivo la protección de la sanidad vegetal es un factor decisivo en la comercialización tanto nacional como internacional. En este sentido, las acciones fitosanitarias que realiza la Dirección General de Sanidad Vegetal facilitan la comercialización de productos vegetales. Cabe señalar que el sector hortícola de México es el más dinámico en términos de su crecimiento, en la generación de empleos y divisas. Aporta en total 23.3 por ciento del valor de la producción del sector agrícola.

Entre las actividades que se realizan en esta Dirección General están la vigilancia del cumplimiento de los niveles adecuados de protección fitosanitaria, ya que de lo contrario las consecuencias afectarían la producción nacional, la calidad del producto, el cierre de mercados y la imposición de barreras fitosanitarias no justificadas arrojando grandes pérdidas económicas.

Otras de las acciones que realiza la Dirección General de Sanidad Vegetal es establecer y dirigir las políticas en materia de prevención, control y erradicación de plagas que afectan a la agricultura; asimismo, se propicia el uso apropiado y sostenible de los recursos naturales, mediante la elaboración e implementación de programas y medidas fitosanitarias, tales como: la constatación, supervisión y evaluación de la condición fitosanitaria de los productos agrícolas, generando de esta manera valor agregado a fin de hacerlos más competitivos en el mercado nacional e internacional.

El complejo de plagas conocidas como moscas de la fruta representa uno de los más serios problemas para la hortifruticultura a nivel internacional. Al respecto, el Gobierno de México tiene una larga historia en el control de moscas de la fruta, por lo que es pionera en algunos de los siguientes temas: a) Uso de la Técnica del Insecto Estéril en área continental para detener el avance de la mosca del Mediterráneo en el Estado de Chiapas; b) Aplicación del concepto de áreas libres de moscas de la fruta; c) Establecimiento de acuerdos cooperativos para la operación regional de programas a gran escala para la erradicación de la mosca del Mediterráneo; d) Construcción y operación de la Planta de Producción de Moscas Estériles (del género *Anastrepha*) y parasitoides más grande del mundo; e) Se cuenta con el Centro Internacional de Capacitación sobre Moscas de la Fruta en Metapa de Domínguez, Chiapas, auspiciado por la Agencia Internacional de Energía Atómica.

La Campaña Nacional contra Moscas de la Fruta se integra por el Programa Mosca del Mediterráneo (con sede en Tapachula, Chiapas); el Sistema de Trampeo Preventivo contra Moscas

Exóticas de la Fruta y el Programa de Control de Moscas de la Fruta del género *Anastrepha*. Estás dos últimas áreas tienen como sede administrativa la Ciudad de México.

Para el Gobierno de México, el Programa Moscamed y el Sistema de Trampeo contra Moscas Exóticas de la Fruta son de primera prioridad, ya que se requiere prevenir la introducción y dispersión de la mosca del Mediterráneo y otras como la mosca oriental de la fruta, mosca del melón, entre otras especies exóticas. El Programa Moscamed tiene carácter Regional entre México, Guatemala y Estados Unidos. Es dirigido en forma trinacional por un Grupo de Alta Dirección de los tres países, cuyo objetivo principal del programa regional es evitar el ingreso de la mosca del Mediterráneo hacia México y Estados Unidos, y avanzar gradualmente en la erradicación de la plaga en Guatemala.

El trampeo preventivo contra moscas exóticas de la fruta tiene como objetivo detectar oportunamente cualquier incursión de estas plagas, además de la moscamed, a territorio nacional para aplicar inmediatamente las medidas de erradicación. Asimismo, demostrar la ausencia de moscas exóticas en México que se asocian a especies hortícolas, tales como: jitomate, diversos cultivares de chiles, sandía, melón, calabacitas, pepino, papa, fresas, entre otras, nos permiten amparar técnicamente las exportaciones de esos productos. Es de resaltar la trascendencia que para el caso del jitomate representa el trampeo preventivo, toda vez que se le vinculan por lo menos 15 especies de moscas de la fruta de los géneros *Bactrocera, Ceratitis, Rhagoletis y Tririthromyia*.

El Programa de Control de las Moscas Nativas de la Fruta (*Anastrepha*) está dirigido a cuatro especies de mayor importancia económica, como la mosca mexicana de la fruta (*A. ludens*), la mosca de las indias occidentales (*A. obliqua*), la mosca de la guayaba (*A. striata*) y la mosca de las sapotáceas (*A. serpentina*). Su objetivo principal es mejorar la competitividad de los sistemas producto mango, guayaba, cítricos dulces (naranja, mandarina y toronja), durazno y manzana, de tal manera que permitan generar valor agregado a los productos agrícolas, mediante la mejora o conservación de estatus fitosanitarios. En este sentido, cada año se exportan en promedio 200 mil toneladas de mango con un valor promedio de 150 millones de dólares.

Como país miembro signatario de la Convención Internacional de Protección Fitosanitaria, México adquiere el compromiso de adoptar las Normas Internacionales de Medidas Fitosanitarias, a efecto de armonizar las medidas fitosanitarias y facilitar la comercialización de los productos vegetales. Es importante señalar que en los últimos tres años se han publicado normas internacionales específicas para el establecimiento y reconocimiento de áreas libres y de baja prevalencia de moscas de la fruta, lo cual refleja la importancia de este grupo de insectos.

Finalmente, un ejemplo más de la importancia de las moscas de la fruta es precisamente ésta Reunión Internacional del Grupo de Moscas de la Fruta del Hemisferio Occidente, bajo el auspicio de la Agencia Internacional de Energía Atómica, donde se presentarán conferencias magistrales por expertos internacionales, así como mesas de trabajo de discusión e intercambio de experiencias en el manejo de Programas Operativos de Moscas de la Fruta en diferentes parte del mundo.

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Sesión/Session I

Manejo de Programas/Management Program

Medfly Program Operational Update: Targeted Suppression of Population Centers of *Ceratitis capitata* Wied. Through the Use of GIS/GPS Technology

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Resume

The Medfly Program was established in 1979 to prevent the unmitigated spread of Mediterranean fruit fly (Medfly), *Ceratitis capitata Wied. (Diptera: Tephritida),* from the generally infested areas of Central America into the Medfly free areas of Mexico, Belize, Guatemala and the United States. Primarily operating throughout Chiapas, the southernmost state of Mexico, and the northern parts of Guatemala, the Program has been successful in maintaining a functional barrier for almost 30 years. However, recent funding reductions have forced program managers to become more creative in the use of available resources. Big budget aerial pesticide application operations are no longer a viable option; the enhanced use of GIS/GPS technology now allows for the identification of population hotspots and the more cost efficient targeting of various mitigation activities.

Introduction

The Medfly Program, a cooperative venture between Mexico, Guatemala, Belize and the United States, is now managed by the Program's Unified Management Team (UMT), which made up of administrative and technical managers from Mexico, Guatemala and the United States. The UMT is responsible for the administrative management and technical leadership of the Program and the oversight of all aspects of field work. For the past ten years the general operating budget of the cooperative program has been about $40 million per year with Mexico and the United States providing most of the funding. This level of funding is used to support survey operations, quarantine enforcement, ground pesticide treatments, public relations, and SIT production and release activities. This normal operating budget does not, however, include funds for the purchase of large amounts of pesticide or the payment for spray planes needed to carry out aerial pesticide applications. In 1998, when huge outbreaks in Chiapas threatened the integrity of the barrier, the United States Department of Agriculture (USDA) stepped in and contributed an additional $10 - $14 million annually in emergency funds for seven years which was used to support a number of large aerial pesticide spray programs, increase SIT production and release of sterile Medflies, and upgrade the Program's infrastructure and facilities. However, the Program stopped receiving these emergency funds in 2006 and, in the face of rising costs in almost all phases of program work, the UMT has now been required to develop alternative strategies to maintain an effective barrier.

Current Strategies

In the area of program work coffee, *Coffea arabica* L., is considered to be the primary host of the Medfly. Several large coffee production belts transverse both Guatemala and Mexico and serve as the primary breeding sites of the wild Medfly population. When the Program had access to the emergency funds large scale pesticide application programs were conducted, often covering more than 100,000 hectares with up to eight applications of first Malathion bait sprays and now more recently Spinosad bait sprays. However the costs of these projects reached up to $10 million a year which is now beyond the reach of the Program. Nevertheless, the situation in the field remains the

same – with wild fly populations fluctuating year to year and often reaching levels of intensity in some specific areas that are beyond control with just the use of SIT releases and ground pesticide applications.

In order to maintain an effective barrier, the Program's technical managers in Guatemala developed a new approach. The Program maintains a network of about 35,000 traps in the field that are all geo-coded and included in a program wide database. Analysis of the survey data over time clearly showed that each year, following the end of the rainy season when the populations densities are normally at their lowest levels of the annual cycle, wild Medfly populations routinely built up first in the coffee production areas and then generally spread throughout the region. The analysis also showed that the fly populations are not uniformly distributed throughout the coffee – rather there are numerous distinct points of high population densities, referred to as "reservoirs," that serve as epicenters for population growth and spread. Using this information the technical managers were able to develop an operational work plan that used targeted spraying (usually only 4 applications) directly on these reservoirs (some blocks as small as 100 hectares) in conjunction with general SIT releases and limited parasitoid releases. The scope of the suppression work was limited to what the Program could afford – in 2007/2008 this amounted to just under $2 million, which allowed for the spraying of only 25,000 hectares in total.

Conclusions

The idea of targeted sprays and low cost spray projects was originally met with great skepticism even within the Moscamed Program. But the results of the first year of work carried out in southwest Guatemala in late 2007 and early 2008 clearly showed that the limited operations were actually highly effective in eliminating the population reservoirs. As a direct result, the general population levels in the neighboring areas were also greatly reduced. It is expected that the annual application of this strategy may be the only way to help maintain the barrier in light of ever shrinking fiscal resources. While this strategy will not lead to eradication, it has shown to be very effective at suppressing populations to the point that SIT and ground sprays alone can complete the work needed to maintain a suppressed population over time. Obviously one year of work is not enough to truly assess the new strategy but the UMT is calling for another round of targeted aerial pesticide applications at the end of this year and perhaps over time we may be able to confirm that this is a cost effective and efficient strategy to keep wild populations suppressed.

Situación Actual de la Campaña Nacional Contra Moscas de la Fruta en México

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Introducción

En 1992 se estableció la Campaña Nacional contra Moscas de la Fruta, con el objetivo de controlar, suprimir y erradicar donde las condiciones agroecológicas lo permitan a cuatro especies de moscas de la fruta de importancia económica (*Anastrepha ludens, A. obliqua, A. serpentina y A. striata),* a efecto de reconocer huertos temporalmente libres, zonas de baja prevalencia y zonas libres de moscas de la fruta.

Para cumplir con la meta, se cuenta con una Planta de Producción Moscas Estériles y Parasitoides, con niveles de producción semanal de 190 millones de *A. ludens*, 40 millones de *A. obliqua,* 50 millones del parasitoide *Diachasmimorpha longicaudata*. Dicho material biológico se distribuye para su empaque y liberación a los estados de Nuevo León, Sinaloa, Nayarit, Zacatecas, San Luís Potosí, Tamaulipas, Baja California, Michoacán y Chiapas.

En este sentido, algunas de las actividades sustantivas para el desarrollo de la Campaña son las siguientes:

- Construcción y acondicionamiento de cinco Centros de Empaque de Adulto en Frío (CEAF) ubicados en Montemorelos, N.L., Cd. Victoria, Tamps., Rioverde, S.L.P., Huanusco, Zac. y El Rosario, Sin., con una inversión de 35 millones de pesos.
- Empaque de pupas en sistemas de Cajas Parc, Torres Worly y Torres México.
- Uso de la hormona juvenil en dos Centros de Empaque (Sinaloa y Nuevo León).
- Liberación del parasitoide *Coptera haywardii* en proyectos pilotos en Chiapas y Nuevo León.
- Expedición de la tarjeta de manejo integrado de moscas de la fruta vía Internet.
- Uso de Sistema de Información Geográfica para el seguimiento de las operaciones de campo y verificación de las liberaciones de moscas estériles y aspersiones en tiempo real.
- Investigación sobre sexado genético de A. ludens para producir una cepa TSL.
- Evaluación externa de la Campaña por un grupo técnico internacional.

Avances

Con relación a los avances, cabe destacar que de 1992 a 2008, se han reconocido como zonas libres de moscas de la fruta 920,570 Km^2 que representan el 47% de la superficie del territorio nacional, donde se cultivan alrededor de 85 mil ha de frutales (cítricos, manzana, durazno y mango). Adicionalmente, se han reconocido 223,763 Km^2 (11.42 % del territorio nacional) como zonas de baja prevalencia de moscas de la fruta en áreas donde se encuentran establecidas 186 mil hectáreas de frutales como cítricos, mango, guayaba y durazno (Figura 1).

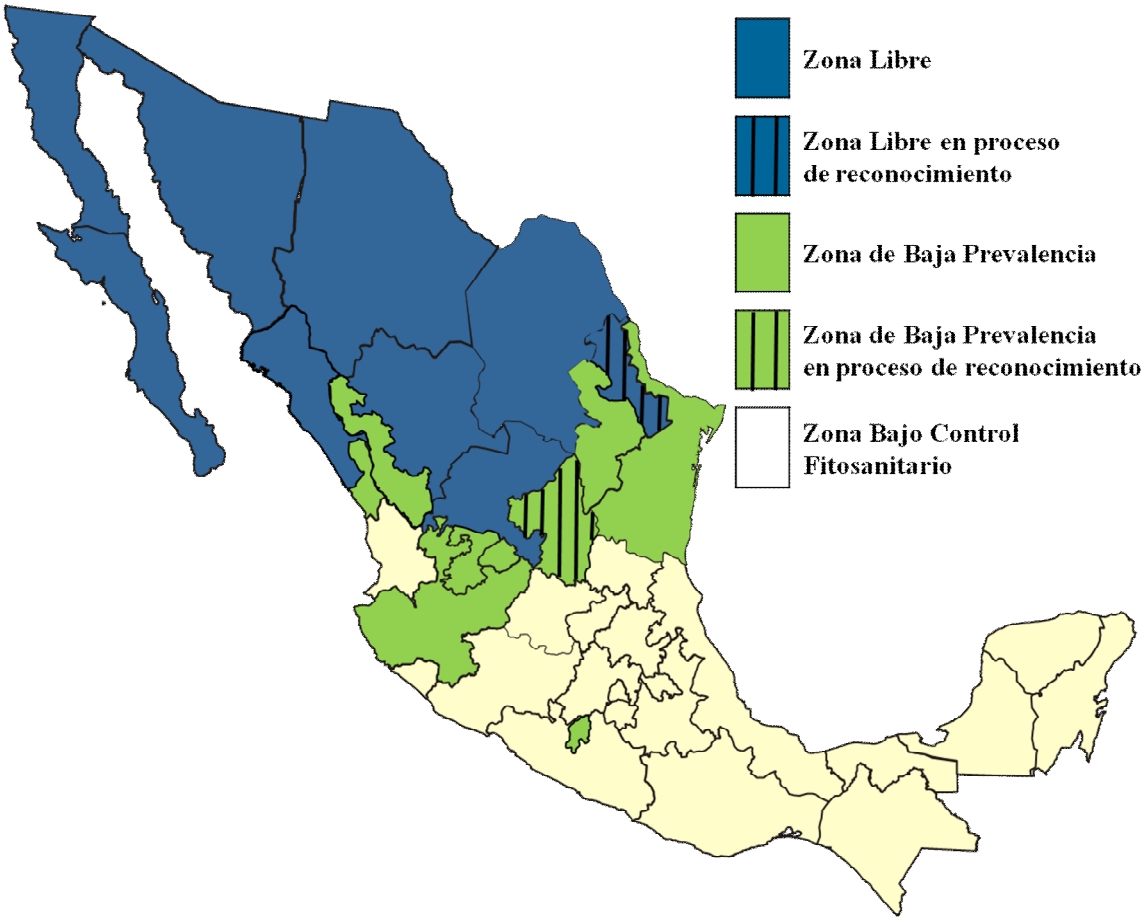

Figura 1. Estatus de Moscas de la Fruta en México (2008**).**

Han sido reconocidos 202,017 km^2 de zonas libres de moscas de la fruta por E.U.A., Australia, Nueva Zelanda, La Unión Europea y Japón.

En zonas libres de moscas de la fruta, para la vigilancia de la plaga se mantienen en operación anual 7 mil trampas McPhail y Multilure, cebadas con proteína hidrolizada o torula. Del 2000 al 2008 se han realizado 2.7 millones de revisiones de trampas. Cuando se detecta la plaga se ejecuta el Plan de Emergencia hasta lograr su erradicación.

En las zonas de baja prevalencia se mantienen en operación anual 8,500 trampas McPhail y Multilure, cebadas con proteína hidrolizada o torula. De 2000 al 2008 se han realizado 2.8 millones de revisiones a las trampas. Se aplicó control químico en 440 mil ha acumuladas, con repeticiones semanales. De la misma manera, se han liberado 400 millones de parasitoides y donde se ha alcanzado la supresión de la plaga se han liberado 10 mil millones de moscas estériles.

En ambas zonas se aplica un control de calidad del trampeo mediante la colocación controlada de especímenes adultos de la plaga con resultados de reporte mayores al 90%. Para la protección de las zonas libres y de baja prevalencia se operan 35 puntos de verificación interna, donde en promedio anual en los últimos diez años se han inspeccionado 10.5 millones de vehículos; se han muestreado 500 ton de fruta; se han decomisado y destruido 300 ton de frutas y se han fumigado 45 mil ton de frutas.

Durante el periodo de 2000 a 2008, de las zonas libres de moscas de la fruta se han exportado 130 mil ton de mango, 113 mil ton de naranja y 5,700 ton de durazno con un valor comercial de 150 millones de dólares americanos. Con relación a la exportación de mango con tratamiento cuarentenario producido en zonas de baja prevalencia y zonas bajo control fitosanitario se han exportado un millón 440 mil ton de mango con tratamiento cuarentenario con un valor comercial de un mil 200 millones de dólares; de igual manera se han exportado 600 mil ton de cítricos con tratamiento cuarentenario de postcosecha con un valor comercial de 500 millones de dólares.

El concepto de Huertos Temporalmente libres de Moscas de la fruta para la movilización nacional sin tratamiento cuarentenario de postcosecha ha tenido un crecimiento notable en los últimos nueve años; a la fecha en 15 estados de la República Mexicana se han certificado 68 mil ha acumuladas de guayaba, durazno, cítricos, mango, granada roja, ciruela, carambola, guanábana, manzana y tejocote.

De 2000 a 2008, el costo de las operaciones de campo de la Campaña Nacional contra Moscas de la Fruta ha sido de 1’710 millones de pesos, de los cuales el Gobierno Federal aportó 660 millones, el Gobierno Federal 450 millones y los productores 600 millones. Para la producción de moscas estériles y parasitoides se han invertido 820 millones de pesos por parte del Gobierno Federal.

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Resúmenes de Posters Presentados/Abstracts of Presented Posters

Sistema Preventivo contra Moscas Exóticas de la Fruta en México

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La exportación de frutas y hortalizas representa para México un importante componente de la economía nacional ya que se cultivan cada año alrededor de 1.7 millones de ha, donde se producen 12 millones de toneladas de productos vegetales con un valor comercial de 4,500 millones de dólares. En este sentido, el Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA) coordina la operación del Sistema Preventivo contra Moscas Exóticas de la Fruta con el objetivo de detectar oportunamente cualquier incursión de la plaga a territorio nacional para su inmediata erradicación. Este sistema consta de 37,130 trampas, de las cuales 21,000 son específicas para *Ceratitis capitata* operadas directamente por el Programa Moscamed en el Estado de Chiapas. En el resto del país se tienen 16,130 trampas para la detección de *C. capitata*, (77%), *Bactrocera dorsalis* (8%), *B. cucurbitae* (8%), *Rhagoletis* spp. (4%) y algunas especies de *Anastrepha* (3%). Las trampas son colocadas en sitios de riesgo potencial de ingreso como son aeropuertos, puertos, centrales de autobuses y rutas carreteras con flujos comerciales. De 1996 a junio de 2008, se han acumulado 5.6 millones de revisiones de trampas, con periodicidad de 14 días, obteniéndose en promedio porcentajes de revisión superiores al 90%. El Sistema Preventivo ha demostrado que el territorio de México está libre de moscas exóticas de la fruta. Únicamente en un área restringida en el Estado de Chiapas, frontera con Guatemala, se detectan especimenes de *C. capitata* en forma aislada y esporádicas y se aplican medidas de erradicación por el Programa Moscamed. En el área productora de olivo en Baja California y Caborca, Sonora está presente *B. oleae*. El costo del Sistema Preventivo contra Moscas Exóticas de la Fruta de 1996 a 2008 ha sido de 21.6 millones de dólares; de los cuales el 84% fue aportado por el Gobierno Federal, 12% por los Gobiernos de los Estados y 4% por los productores.

Supresión de la Mosca del Olivo *Bactrocera oleae* (Diptera: Tephritidae) en Caborca, Sonora, México

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En octubre de 2000 se detectó por primera ocasión la mosca del olivo (*Bactrocera oleae*) en el Municipio de Caborca, donde se tienen establecidas 2,000 ha de olivo. En 2001 se inició la

supresión de la plaga con el objetivo de reducirla a niveles indetectables. La Junta Local de Sanidad Vegetal de Caborca (JLSVC) realiza el trampeo y muestreo de frutos; los productores recolectan la aceituna en la etapa de postcosecha, estableciéndose como fecha límite el 30 de noviembre; en caso contrario, la JLSVC contrata técnicos con recursos de los productores que entregan a dicha junta local como garantía de cumplimiento (200 dólares/ha). Adicionalmente, dicha Junta realiza el control mecánico y químico en áreas marginales. El Comité Estatal de Sanidad Vegetal de Sonora aplica la regulación cuarentenaria en el Punto de Verificación Interna de San Luís Río Colorado, en los límites con el Estado de Baja California. De 2002 a julio de 2008, en una red permanente de 500 trampas multilure, cebadas con proteína hidrolizada, con más del 95% de trampas revisadas cada semana y 185 mil revisiones acumuladas, se observa una reducción notable de la plaga de 7,743 a 82 especimenes adultos; y con base en el índice MTD de 2008 que es de 0.0006 y el hallazgo de un foco larvario en 2007 se puede concluir que la mosca del olivo se encuentra bajo supresión. Durante 2007 se cosecharon 9,500 ton de aceituna; de las cuales 9 mil ton se exportaron en fresco a Estados Unidos y 500 ton se enviaron a la industria, con un valor comercial de 6 millones de dólares. El costo del programa de supresión de 2002 a 2008 fue de 2.46 millones de dólares; de los cuales, el Gobierno Federal invirtió 40%, el Gobierno del Estado de Sonora 27% y los productores 33%.

Sistema Integrado Información de Moscas de la Fruta del Perú

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Es un software desarrollado a partir del año 2000 por la Subdirección de Moscas de la Fruta y Proyectos Fitosanitarios del SENASA - Perú, esta trabajado en lenguaje de programación Power Builder, tiene como plataforma de base de datos a Oracle, registra data técnica generada por las actividades relacionadas a proyectos de control, supresión y erradicación de moscas de la fruta, con utilización de Sistemas de Posicionamiento Global y está integrada con Sistemas de Información Geográfica mediante una Red WAN e intranet. Actualmente se encuentra operando a nivel nacional y permite entre otras cosas conocer el status de la plaga en el país On Line. A través de este sistema se realiza el monitoreo y supervisión de las actividades de Registro de Productores, Hospedantes y Especies de Moscas de la Fruta, Vigilancia, Control Integrado, Identificación Taxonómica, Administración de Áreas Libres, Programas de Exportación y seguimiento de Personal. Asimismo contamos con una red de estaciones meteorológicas automatizadas a nivel nacional cuyos datos ingresan a nuestro sistema en forma regular. El software permite la generación de reportes tanto tabulares, gráficos y de mapas temáticos, muy importantes para la adecuada toma de decisiones con información en tiempo real, que sea oportuna, confiable y veraz. El software integra un módulo que de Análisis Predictivo de Riesgo para Moscas de la Fruta, en base a un modelo matemático elaborado sobre la base de datos del Sistema Integrado de Información y el ciclo biológico del insecto. Realiza el envío de reportes sobre Moscas de la Fruta a Productores en forma regular y automatizada vía correo electrónico.

The Eradication of an Outbreak of Carambola Fruit Fly in State of Pará, Brazil

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The carambola fruit fly (CFF), *Bactrocera carambolae,* is a pest of many tropical fruit species as carambola, jambo, guava, mango and Antilles cherry. This is a quarantine species for Brazil. Presently the species is found in the State of Amapa and is under active phytosanitary control. The spread out of this pest in the Brazilian territory represents direct fruit losses, considerable increase in the use of insecticides and losing markets in countries with quarantine barriers. On Feb 12, 2007, three males of CFF were captured in traps in the MAPA detection system in the district of Monte Dourado in the Jari River Valley, in the state border Amapa-Para. The detection of a single adult is trigger for immediate application of the Contingence Plan for Eradication of Carambola Fruit Fly, approved and in place by MAPA and by the Agrihealth State Agency of State of Para (ADEPARA). The team responsible for the survey and control actions in the Contingency Plan has 28 people including the sanitary education core. For the actions were used 6 trucks. The control actions following the work plan were: distribution of 67,740 impregnated blocks and malathion as male annihilation technique, MAT ; application of 3,387 liters of protein bait spray applied in host plants; collection of 4,876 kilos of fruit host; elimination of 850 carambola trees. After the continuous application of phytosanitary measures in Monte Dourado, the last capture of an adult was on Jun 8, 2007 and a larva in fruit was on Jul 20. In Laranjal the last adult capture was on Aug 13 and no capture of larva up to date. Hence, the survey demonstrate that CFF is absent in the area for a period over five life cycles. As the period of time since the last detection exceeds three life cycles of the target species, the outbreak was declared eradicated on Feb 29, 2008.

Sanitary Education Actions as a Component of a Carambola Fruit Fly Outbreak Eradication in Jari River Valley, Brazil

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An outbreak of *Bactrocera carambolae* was detected in Feb 2006 in district of Monte Dourado, State of Para, Brazil. The district is located just across of the city of Laranjal do Jari separated by the Jari River in State of Amapa. Hundreds of workers living in Laranjal commute everyday to Monte Dourado bringing continuous risk of infested fruit from Amapa to Para. Sanitary education measures are more effective that regulatory ones and helps the contingency plan to disseminate the importance of program and reduce the complains with the control measures applied in program. Also, having in account the area wide concept, both cities as state borders should be targeted. In such remote area, where there are strong constrains in terms of communication and transportation with local communities, sanitary education is a key element. In this regard, Ministry of Agriculture

built partnership with Environmental agencies, Municipalities Boards, Community Association in order to have deeper involvement of the population. Sanitary education actions and public relation targeting the residence human population were carried out during the eradication measures. The actions were done either in the outbreak area as the surrounds in order to have the population more sensitive concerning the measures that have forbid fruit transportation and trade and eradication actions. The adopted method for training was SOMA method that was shown very adequate for reaching the goals. The project was recognized as one of most important innovative initiative countrywide in 2007. The Sanitary Education Core has prepared 300 multipliers that have trained 3,248 elementary, middle and high school students and support 872 families. The actions were carried out in the Jari River Valley were considered crucial to enforce the regulation in an area where the communication is very difficult and the main transport is through the boats in the river system. Dissemination material such as folders, booklets, t-shirt was prepared and the impact of the actions was evaluated as long lasting.

Development of Worldwide Tephritid Fruit Fly Networks Through Tephritid Workers Databases (www.tephritid.org)

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Tephritid Workers Database (TWD) **(www.tephritid.org)** is a web-based database collecting and sharing information on tephritid fruit flies, one of the most economically important groups of insect species that threaten fruit and vegetable production and trade worldwide. Each year, a significant amount of information is made available on tephritid biology, ecology, taxonomy, biotechnology, control methods, outbreaks, and operational control programs. In parallel, an increasing number of scientists and other stakeholders with various expertise are involved in these research and control activities worldwide. In this regard, it was considered important to develop a global database in order to allow tephritid fruit fly workers worldwide to keep up-to-date on the most recent developments, to provide an easily accessible and always available resource, and to serve as a platform of interaction that promotes collaboration and communication among scientists. Launched in 2004 on the internet, TWD includes a Directory of Tephritid Workers that has now more than 880 members from more than 100 countries; news; a bank of more than 1900 recent bibliographic references on fruit flies; and relevant internet links on fruit flies. TWD is self-maintained by the members and depends on their active contribution such as regularly updating their records and publication list, or posting relevant news, alerts, urgent messages, job opportunities, new books, new publications, and web site links. Alongside, TWD provides an advanced search tool to facilitate data retrieval, within the database sources, per countries, subjects, fruit fly genera, authors and literature. As a neutral service for the worldwide scientific community, TWD offers a platform also for other regional Tephritid subgroups such as The Tephritid Workers of Europe, Africa and Middle East (TEAM) and efforts are being undertaken to integrate other regional subgroups such as the Working Group on Fruit Flies of the Western Hemisphere.

Sistema de Detección Temprana para la Mosca del Mediterráneo en el Programa Regional Moscamed Mexico-Guatemala

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Desde el año 2,000 el Programa Regional Moscamed México-Guatemala-Estado Unidos ha utilizado cinco tipos de trampas para la detección de la mosca del Mediterráneo *Ceratitis capitata* Wied.: trampas Fase IV y Multilure cebadas con BioLure (Acetato de Amonio [AA], putrescina [PP] y trimetilamina [TMA]), y trampas Jackson, Panel amarillo y C&C cebadas con TrimedLure. Estos sistemas de trampeo fueron desarrollados a través de los programas coordinados de investigación (PCI) del OIEA (OIEA 1996, OIEA 1998). Se realizó un análisis de la información generada en los años 2007 y 2008 para determinar si la red de trampeo a gran escala del Programa Regional Moscamed, mantiene el objetivo de detección temprana para los brotes de la mosca del Mediterráneo. La red de trampeo en Chiapas en el área libre está compuesta por 14,000 trampas en proporción 1:1 trampas$^{EXA®}$ con BioLure$^{®}$:trampas$^{EXA®}$ con TrimedLure$^{EXA®}$; y en el área de baja prevalencia con 5,000 trampas en una proporción de 4:1 trampas$^{EXA®}$ con Biolure$^{®}$:trampas$^{EXA®}$ con TrimedLure$^{EXA®}$. El análisis de la información indicó que hay una diferencia mayor en Moscas por Trampa Atendida al Año (MTA), para las trampas con Biolure. También estas trampas capturaron el 84% de las moscas fértiles en 2007 y 80% en 2008. En área de Baja Prevalencia en 2007 la captura de moscas fértiles se elevó a 91% en trampas con Biolure. Resalta a la vista la diferencia en la captura de hembras para las trampas con BioLure, arriba de 8 veces en 2007 y 9.5 veces en 2008, así como la captura del 56% de hembras vírgenes-jóvenes (1-7 días de edad), lo que corrobora una detección temprana de brotes. Con estos resultados se respalda la decisión del Programa Moscamed, asesorado por su Technical Advisory Committe (TAC), para que en el área libre de Chiapas, se instale también la proporción de 4:1 a partir del año 2009, buscando incrementar aún más la detección temprana de brotes de la plaga**.**

Sistema de Geolocalización (GPS) y Administración de la Información (GIS) en Línea (Web) Ligados en un solo Sistema llamado T-SIGA, para las Operaciones de Campo del Programa Regional Moscamed Chiapas-México y Guatemala.

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El sistema T-SIGA ha sido desarrollado con hardware y software acorde a las necesidades del Programa Regional Moscamed. La información básica de georeferencias, tipo de trampa, hospedero, fechas, hora, con captura de moscas del Mediterráneo o sin captura, reubicaciones, etc., que acompaña a cada trampa instalada y revisada, se codifica en el sistema de barras. La información almacenada es actualizada por una computadora digital personal (PDA-GPS) y esta información es transferida a la Web para alimentar la base de datos interactiva en el servidor y portal dedicado para este fin. A través del GIS se maneja la base de datos, para limpiar, filtrar, integrar, almacenar, recuperar, analizar, desplegar y visualizar la información espacial en mapas. El

T-SIGA contribuye además al seguimiento de la ejecución de las rutas de trampeo, tiempo y distancia del recorrido, hora de revisión y periodo de exposición de cada trampa, cambio de materiales y atrayentes, etc. Por el T-SIGA se opera y maneja la información que genera la revisión de la red de trampeo (20,000 trampas) y facilita la toma oportuna de decisiones y estrategias a implementar en la detección y control de la mosca del Mediterráneo y el uso de la Técnica del Insecto Estéril. El sistema T-SIGA está iniciando la operación de área "wide" en Chiapas (75,000 km^2) y se extenderá a Guatemala en 2009 para abarcar las todas las áreas regionales de trabajo (152,200 km^2 y 30,000 trampas).

Sesión/Session II

Taxonomía, Biología y Genética/Taxonomy, Biology and Genetics

Estado Actual de la Taxonomía del Género *Anastrepha* Schiner

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Introducción

El género *Anastrepha* Schiner constituye el género más grande y de mayor importancia económica en América. En la última revisión del grupo hecha por Norrbom *et al*. (1999) se enlistaron un total de 197 especies válidas, estimando que aun existen alrededor de 50 especies más por describir. Algunas publicaciones posteriores a esa fecha han incorporado descripciones de nuevas especies (Norrbom 2002, Norrbom *et al*. 2002 y 2005, Norrbom y Caraballo 2003, Hernández-Ortiz 2004, Tigrero 2006 y 2007, Tigrero y Salas 2007, Norrbom y Korytkowski 2007), así como algunos nuevos registros para Colombia (Martínez-Alava 2007).

El conocimiento de la diversidad de especies a nivel país ha sido analizada recientemente sólo en ciertos casos como Venezuela (Morales y González 2007), Brasil (Zucchi 2000, Zucchi 2007), y México (Hernández-Ortiz 2007). Con base en esta información, aquí se presenta una lista actualizada de *Anastrepha*, que incluye un análisis global de su distribución a partir de los grupos de especies presentes en las diferentes áreas biogeográficas, y los datos registrados para cada uno de los países del continente Americano.

Taxonomía

De acuerdo con la información taxonómica basada en la morfología, el género *Anastrepha* comprende en la actualidad un total de 216 especies válidas clasificadas en 18 grupos de especies. En estos grupos se concentra el 87% de las especies conocidas, mientras que el 13% restante aun permanecen sin una clasificación precisa en grupo alguno. De todos los grupos reconocidos, sólo 8 de ellos han sido revisados taxonómicamente (*Benjamini*, *Caudata*, *Daciformis*, *Doryphoros*, *Grandis*, *Hastata*, *Schausi* y *Serpentina*), en los cuales se incluyen poco menos del 25% de las especies totales. Por el contrario, los grupos más diversos como *Fraterculus* (33 spp), *Mucronota* (32 spp) y *Pseudoparallela* (20 spp), aun permanecen sin una revisión taxonómica integral que nos permita precisar las relaciones entre sus especies, y particularmente en el primero de ellos, en donde ocurren las especies nativas de América de mayor importancia económica.

Las relaciones filogenéticas de *Anastrepha* Schiner lo ubican como un género hermano de *Toxotrypana* Gerstaecker y *Hexachaeta* Loew dentro de la tribu Toxotrypanini, cuyos miembros poseen una distribución exclusivamente Neotropical, a lo largo de las regiones tropicales y subtropicales del continente americano (Norrbom *et al*. 1999, Hernández-Ortiz 2006).

Biogeografía

Por las características de su distribución continental, el género *Anastrepha* presenta un patrón de dispersión "Neotropical Típico" (*sensu* Halffter 1976), caracterizado por elementos originados en el sur del continente y distribuidos a lo largo de las tierras bajas tropicales americanas, pero con limitaciones de penetración en el norte del continente, debido principalmente a factores ecológicos y geográficos.

Siguiendo la biogeografía de América Latina basada en su entomofauna (*sensu* Morrone 2001, 2006), la región Neotropical se encuentra divida en cuatro grandes subregiones: a) Caribeña, conformada por los dominios Mesoamericano, Nor-Oeste Sudamericano y Antillano; b) subregión Amazónica; c) subregión Paranaense; y d) subregión Chaqueña.

El análisis de la distribución de especies basado en la clasificación supra-específica del género *Anastrepha*, mostró que siete grupos de especies pueden ser considerados de amplia distribución, ya que poseen representantes en todas las áreas biogeográficas de la región Neotropical: *Fraterculus*, *Mucronota*, *Pseudoparallela*, *Daciformis*, *Serpentina*, *Spatulata*, y *Dentata*. Por el contrario, los grupos restantes presentan patrones de distribución restringida a ciertas regiones (ver Cuadro 1).

Cuadro 1. Análisis de la distribución regional de los grupos de especies del género *Anastrepha* basado en las principales áreas biogeográficas de la región Neotropical.

Anastrepha Grupos de Especies	No. spp.	dominio Antillano	dominio Meso-Americano	dominio Nor-Oeste SudAmericano	sub-región Amazónica y Paranaense	sub-región Chaqueña
Fraterculus	33	5	13	19 (5)	19 (7)	6 (2)
Mucronota	32	6 (4)	4	17 (9)	16 (7)	2
Pseudoparallela	20	2	2	13 (7)	10 (2)	6 (2)
**Daciformis*	13	4 (4)	4 (3)	3	4	3
Robusta	11		1	6 (2)	7 (4)	
**Serpentina*	11	3	3	9 (4)	5	1
Spatulata	11	1	6	5	7	6
Dentata	10	3 (2)	3 (2)	2	6 (3)	1
Cryptostrepha	8		5 (3)	5 (2)	1	
**Grandis*	8			6 (3)	5 (2)	1
Leptozona	8	1	2	6 (4)	4 (2)	
**Schausi*	5		1	3 (3)	1	
**Benjamín*	4			3 (3)	1	
Punctata	4			1	4 (2)	2
**Caudata*	3			2	3	
**Hastata*	3		2	2	1	
**Doryphoros*	2			1	1	
Ramosa	2			2 (2)		
Incertae sedis	28	2	3	13	19	2
Total especies	**216**	**27**	**49**	**118**	**114**	**30**
Especies Endémicas	**133**	**13**	**13**	**51**	**49**	**7**
* Grupos de especies revisados						
( ) Grupos con más de una especie endémica en la región						

El dominio Antillano, comprende las islas Mayores y Menores del Mar Caribe, así como la porción sur de la península de La Florida (EUA). En ese dominio se reconoce la existencia de 27 especies, sin embargo, los datos regionales muestran la fauna Antillana aun no está bien estudiada,

debido a que casi la mitad de las especies registradas sólo se conocen para la isla de Trinidad, la cual mantiene una mayor influencia de especies de origen continental (e.g., *A. leptozona, serpentina, striata, antunesi, distincta* y *fraterculus*). En cambio, países como Cuba, Jamaica, Puerto Rico, Rep. Dominicana y Haití, sólo registran entre 4 y 8 especies en cada uno de ellos. A pesar del conocimiento parcial de las faunas de esos países, el dominio Antillano posee 13 especies endémicas entre las que se encuentra *A. suspensa*.

El dominio Mesoamericano ocupa gran parte del territorio Mexicano y América Central hasta Costa Rica, así como la parte sur de Texas (EUA) como una extensión de la "Zona de Transición Mexicana". En este dominio se conocen 49 especies, pero sólo dos países en la región se encuentran bien documentados, México (37 spp) y Costa Rica (32 spp); en caso contrario, los países restantes de América Central poseen escasos registros, por lo que aun son necesarios estudios a nivel país. El dominio Mesoamericano contiene en sus territorios 13 especies endémicas.

El dominio Nor-Oeste Sudamericano comprende diversos territorios de países como Panamá, Venezuela, Colombia y Ecuador. Aquí han sido registradas un total de 118 especies, por lo cual debe ser considerada la zona de mayor diversidad, aun cuando sólo Panamá (69 spp) presenta un conocimiento taxonómico aceptable, en contraste con las faunas de países como Venezuela (55 spp), Colombia (36 spp) y Ecuador (35 spp), cuyas áreas de influencia amazónica no han sido bien estudiadas. Los análisis biogeográficos preliminares de esta región indican la presencia de 51 especies endémicas, lo cual reitera la necesidad de estudios posteriores.

La sub-región Amazónica (ocupada principalmente por territorios de Brasil, Las Guyanas, Surinam, y algunos territorios de Perú y Bolivia), y la subregión Paranaense (representada por la porción Sureste de Brasil a lo largo de la costa Atlántica), en ambas se localizan 114 especies de las cuales 49 constituyen endemismos. Sin embargo, de todos esos territorios, solo Brasil podría ser considerado como el país mejor conocido con 98 especies, que representan cerca del 45% del total de las especies conocidas, mientras que las regiones amazónicas de Perú y Bolivia han sido pobremente estudiadas. En estas regiones pocas inferencias podemos hacer acerca de su biogeografía, debido a que se requiere información a nivel regional para reconocer cuales especies podrían tener una afinidad exclusivamente Amazónica o Paranaense.

Por último, en la sub-región Chaqueña representada principalmente por los territorios de Paraguay, Uruguay, norte de Argentina y una porción del centro y noreste de Brasil, ocurren un total de 30 especies, casi todas ellas presentes sólo en Argentina (29 spp). En esta sub-región se encontraron 7 especies endémicas (*A. schultzi, repanda, rosilloi, irradiata, pastranai, mburucuyae* y *umbrosa*), pues representa el extremo sur de la distribución natural de *Anastrepha*, y estária considerada como el área biogeográfica menos diversa.

Considerando el total de especies descritas para *Anastrepha*, el análisis biogeográfico preliminar mostró que sólo la sub-región Amazónica + Paranaense, y el dominio Nor-Oeste Sudamericano poseen representantes de todos los grupos de especies: la primera con el 52.7% de las especies conocidas, incluyendo 49 especies endémicas; y la segunda que registró el 54.6% de las especies, incluyendo 51 especies endémicas. En cambio, en el dominio Mesoamericano solo ocurre el 22.7% de las especies representando la distribución más norteña del continente, de las cuales 13 son endémicas para la región; mientras que las áreas menos diversas resultaron ser el dominio Antillano (12.5%) y la sub-región Chaqueña (13.8%), ambas con 13 y 7 endemismos, respectivamente.

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Morphological Characters and Rapid Identification Methods of Fruit Fly Larvae (Diptera: Tephritidae)

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Larvae of fruit fly have the primary responsibility in causing the greatest damage to fruits. Also, they are responsible for most of fruit rejections when they are detected in inspection sites, point of entry or destination. However, larvae of fruit flies have not been used properly in systematics. At least there are 3 reasons for this fact: a) Larvae have few sclerotized and visible characters, b) with the great advance in the techniques of molecular genetics, classical taxonomic studies have lost interest among young researchers, c) in fruit fly collections there are no or very few properly identified individuals for study. Due to the facts mentioned above, currently there are very few specialists in taxonomy of immature stages. Therefore, at present we are at a stage where morphological studies of larvae are very insufficient, focusing primarily on the third instar larvae (Steck *et al.* 1990, White and Elson-Harris 1992, Frías *et al.* 2006) In addition, there is not a common methodology and it has not been emphasized those characters that could be useful in the rapid identification of species, especially those of economic importance. This paper examines the morphology of the third instar larvae of 7 species of the genus *Anastrepha* from Mexico (*A. obliqua, A. striata, A. ludens, A. serpentina, A. cordata, A. crebra* and *A. suspensa*) with the purpose to facilitate their identification, comparing them with populations of *A. fraterculus* and *A. leptozona* already studied.

Some specimens were macerated overnight in 10% solution of cold KOH. The cephalopharyngeal skeleton was dissected and put into glycerin, then being observed and characterized by means of an optical microscope (OM). Figure 1 shows the main characters analyzed in the cephalopharyngeal skeleton.

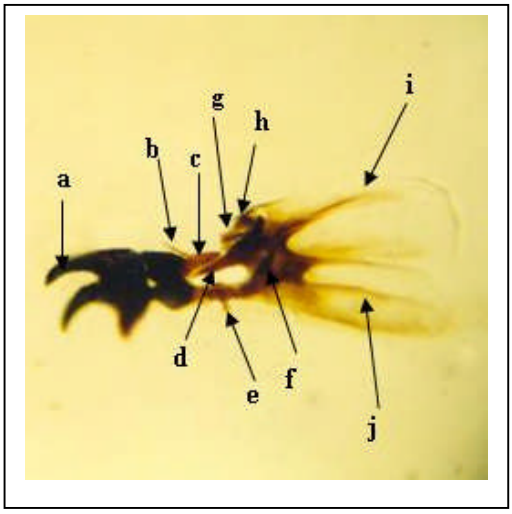

Figure 1. Cephalopharyngeal skeleton of third instar larva of *A. suspensa*. a = Apical tooth; b = parastomal bar; c = hypopharyngeal sclerite; d = Basal bar of pharyngeal sclerite; e = Ventral bridge of pharyngeal sclerite; f = Pharyngeal sclerite; g = Anterior sclerite; h = Dorsal bridge of dorsal cornu; i = doral cornu; j = Upper bar of ventral cornu.

Other larvae were prepared for Scanning Electron Microscope (SEM) and the morphology of the following structures were studied: oral ridges, preoral organ, labium shaped, labium sensilla, antennomaxillary complex, anterior and posterior spiracles, spinules, sensilla of the caudal segment, and anal lobes. Terminology used follows Carroll and Wharton (1989), White *et al.* (1999) and Frías *et al.* (2006).

Body length of *A. crebra* larvae is lower than the other species studied. All species studied are different in their cephalopharyngeal skeletons morphology, in particular: sclerotization, labial sclerite, dorsal bridge of cornua, ventral bridge of pharyngeal sclerite. Besides morphological differences were found at: width of hypopharyngeal sclerite and cornua in dorsal view, length and sclerotization of anterior sclerite, upper bar of ventral cornua, basal bar of pharyngeal sclerite (Table 1).

Table 1. Differences in body larvae length, wide of hypopharyngeal sclerite in dorsal view, length and sclerotization of anterior sclerite, shaped of upper bar of ventral cornua and basal bar of pharyngeal sclerite (at OM).

Species	**Body length (mm)**	**Width of hypopharyngeal sclerites. Dorsal view (mm)**	**Length and sclerotization of anterior sclerite**	**Upper bar of ventral cornua**	**Basal bar of pharyngeal sclerite**
A. cordata	7.5-10.0	>0.15	Do not exceed the dorsal bridge of cornua. Weakly sclerotized	Curved and wide in the middle	Long and slender
A. crebra	6.0-7.0	<0.15	Do not exceed the dorsal bridge of cornua. Weakly sclerotized	Curved and wide	Short and wide
A. fraterculus	8.0–10.1	>0.15	Exceed the dorsal bridge of cornua. Strongly sclerotized	Straight and slender	Long and slender
A. leptozona	7.0-11.0	>0.15	Do not exceed the dorsal bridge of cornua. Weakly sclerotized	Straight and slender	Long and slender
A. ludens	9.0-11.0	>0.15	Exceed the dorsal bridge of cornua. Strongly sclerotized	Straight and slender	Long and slender
A. obliqua	9.0–10.0	>0.15	Exceed the dorsal bridge of cornua. Strongly sclerotized	Straight and slender	Long and slender
A. serpentina	9.1–11.0	>0.15	Do not exceed the dorsal bridge of cornua. Strongly sclerotized	Straight and slender	Long and slender
A. striata	8.0-11.0	>0.15	Do not exceed the dorsal bridge of cornua. Weakly sclerotized	Straight and slender	Short and slender
A. suspensa	6.7–9.0	>0.15	Exceed the dorsal bridge of cornua. Strongly sclerotized	Straight and slender	Long and slender

Other morphological differences in the following characters can be observed at SEM. All the species are different in the morphology of: oral ridges, anterior and posterior spiracles, labium, spinules and anal lobes. Also we described differences in presence or absence of papilla sensillum at the base of the distal segment of the antenna, presence or absence of papilla sensilla and pits in the labium, morphology and number of peg sensilla of preoral organ, length and wide of tubules of anterior spiracles (Table 2).

Table 2. Differences in morphology of tubules of anterior spiracles, number of labium sensilla, presence or absence of papilla sensilla close to distal segment of the antenna and number of preorgan conical peg sensilla (at SEM).

Species	Tubules of anterior spiracles	Labium sensilla	Papilla sensilla close to distal segment of antenna	Anal lobes	Number of preorgan conical peg sensilla
A.cordata	As long as wide	2 papilla sensilla and 2 pits	Presence	Unilobated, rounded	3
A.crebra	Longer than wide	2 papilla sensilla	Absence	Unilobated, rounded	1
A.fraterculus	Longer than wide	Not evident	Absence	Unilobated, rounded	3
A.leptozona	Longer than wide	2 papilla sensilla	Absence	Unilobated, rounded	6
A.ludens	Longer than wide	2 papilla sensilla and 2 pits	Absence	Bilobated, rounded	3
A.obliqua	Longer than wide	4 papilla sensilla	Absence	Unilobated, pear shape	5
A.serpentina	Longer than wide	2 papilla sensilla and 2 pits	Absence	Bilobated, rounded	8
A.striata	As long as wide	2 papilla sensilla and 2 pits	Absence	Bilobated, rounded	11
A.suspensa	Wider than long	4 papilla sensilla	Absence	Unilobated, rounded	8

All larvae studied here can be identified according to the differences detected in the cephalopharyngeal skeleton. The identification of each species can be confirmed through characters observed through the SEM.

It is important to have a collection of larvae of different developmental stages of each species presenting pictorial keys to help their identification. It proposes a protocol for the collection, dissection and preservation of larvae. It is also important to train specialists in taxonomy of immature stages of fruit flies in order to resolve specific problems in each country. These studies must be complemented by those made at the molecular level.

Acknowledgements

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Incipient Speciation Revealed in *Anastrepha fraterculus* by Studies on Mating Compatibility, Hybridization and Cytology

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Introduction

Reproductive isolation among different populations of the *Anastrepha fraterculus* (Diptera: Tephritidae) Wiedemann species complex has been found acting both at the pre- and post-zygotic levels (Selivon *et al.*, 1999, 2005; Vera *et al.*, 2006). Recently major differences were shown in behaviour, cytology, biochemistry and genetics between two populations from Argentina and Peru (Cáceres *et al.*, 2008) resulting in high levels of reproductive isolation. In field cage choice tests, hybrid males could mate with females of either parental strain. However in these tests no hybrid females were used. Differences in pheromone blends between the parental strains and their hybrids, suggested that hybrid females may mate preferentially with hybrid males. Differential location of the calling males from Argentina and Peru in the tree may also contribute to the pre-mating isolation. This paper provides information on these two aspects.

Material and Methods

Strains. For details of the parental strains see Cáceres *et al.* (2008). H_{AP} are hybrids obtained from the cross between Argentina males and Peru females while H_{PA} are hybrids from the reciprocal cross.

Field cage mating tests. Experimental protocols and fly handling followed Cáceres *et al.* (2008). The location and origin of flies in mating pairs were noted. Location in the tree was assigned as: upper, middle and low and north, east, south and west.

Bisexual test with parental strains. 25 flies from each sex from the parental strains.
Unisexual test with parental strains: 25 males from the parental strains with either 25 females from Argentina or 25 females from Peru.

Unisexual test with hybrid males: 25 males and 25 females from one of the parental strains with 25 males from one of the hybrids (either H_{AP} or H_{PA}).

Unisexual test with hybrid females: 25 males and 25 females from one of the hybrids (either H_{AP} or H_{PA}) with 25 males from one of the parental strains.

Data analysis. The distribution of mating pairs in the tree was analyzed using χ^2 test of homogeneity. Mating compatibility was determined using the Index of Sexual Isolation (Cayol *et al.*, 1999) and departures from zero (indicating non random mating) were evaluated using χ^2 test of Goodness of Fit.

Results

In the bisexual tests, matings with Argentina males were found more frequently in the top and SE part of the tree, while matings with Peru males were more evenly distributed in height and tended to be found in the NW quadrant (Fig. 1a). These differences were significant, $\chi^2_2 = 14.39$; P = 0.001; and $\chi^2_3 = 8.87$; P = 0.031, for height and quadrant respectively. In the unisexual tests with parental strains, there was only a significant difference in height ($\chi^2_2 = 7.54$; P = 0.023) (Fig. 1b).

In the unisexual tests with the hybrid males, there were no differences in the distribution of the mating males, except between Argentina and H_{AP} males ($\chi^2_2 = 6.30$; P = 0.012). Matings with H_{AP} males were more evenly distributed than those with H_{PA} males and they tended to avoid the northern quadrant. Matings involving H_{PA} males were found more frequently in the top of the tree and were concentrated in the eastern quadrant (Fig. 1c). These differences were significant, $\chi^2_2 = 6.30$; P = 0.012; and $\chi^2_3 = 8.79$; P = 0.032, for height and quadrant, respectively.

Mating activity in the tests with hybrid females was in general high indicating good conditions (Table 1). Mean ISI were always above zero (Table 1) indicating that hybrid females mated more frequently with hybrid males. The number of matings that involved hybrid males was always higher than the expected under random mating.

Table 1. Mean percentage of mating and mean ISI (both with their associated standard error) for each type of test with hybrid females. The results of the χ^2 test of goodness of fit are also presented.

Type of test	% Mating (SE)	ISI	χ^2	p-value
H_{AP} – ARG	81.50 (2.92)	0.27 (0.07)	11.344	<0.001
H_{AP} – PERU	75.00 (2.24)	0.42 (0.05)	25.627	<0.001
H_{PA} – ARG	79.00 (3.44)	0.15 (0.06)	4.278	0.038
H_{PA} – PERU	73.00 (4.46)	0.29 (0.06)	14.769	<0.001

Discussion

Males from the two populations mated in different places on the tree. The position where mating couples are found is a reliable indicator of the position of males during pheromone calling (Segura *et al.* 2007) and may indicate the occurence of mixed leks. Although both types of male preferred the highest portion of the tree, the Argentina males tended to be the higher. This could be related to the time of sexual activity for the Argentina males which was early in the morning when the temperature in the cages was around 18-22°C, but the Peru males reached peak sexual activity when the temperature was higher, sometimes near 30°C at the top of the cage, making intermediate heights of the tree a more suitable or attractive place to form a lek.

Argentina males were also found more frequently in the northern or eastern quadrants than Peru males. This part of the cage received the first direct sunlight, and the fact that the males aggregated where the light hits first was also noted in other studies (Malavasi *et al.*, 1983; Segura *et al.*, 2007). Although these differences were not detected in the unisexual tests, the proportion of males in each quadrant shows the same trend: Argentina males aggregated in the NE sector. Peru males mated later than Argentina males when light conditions were more homogeneous in the tree and this may explain why the distribution of the Peru males was more uniform.

Hybrid males did not show differences with the parental males regarding location in the height of the tree, except between H_{AP} and Argentina males. H_{AP} males were more evenly distributed between an intermediate height and the top of the tree, while H_{PA} males tended to call

more frequently from the top of the tree. Although the distribution among quadrants was very similar between hybrid males, H_{AP} males had a more even distribution while H_{PA} tended to avoid the northern quadrant. These results suggest that the location of the hybrid in the tree depends on maternal lineage, since H_{AP} males behave as Peru males and H_{PA} males as Argentina males.

Argentina and Peru males have different pheromone blends (Cáceres *et al.*, 2008) and this could play a part in sexual isolation. However, the male hybrids show an almost identical pattern of pheromone composition, being a mix of the parental profiles enabling hybrid males to successfully compete with parental males. However, if hybrid females only respond to pheromone blends that contain compounds from both parental strains there would be a higher mating success with hybrid males in comparison with that of parental males. This is what was found in the field cage tests: hybrid females showed a significant preference to mate with a hybrid males than with parental males.

These results suggest that if these populations hybridized in the field, the hybrid females would tend to mate with hybrid males and this could then lead to the formation of a new entity within the *A. fraterculus* complex. This simple and fast process could be one reason for the high number of taxonomic entities within this complex.

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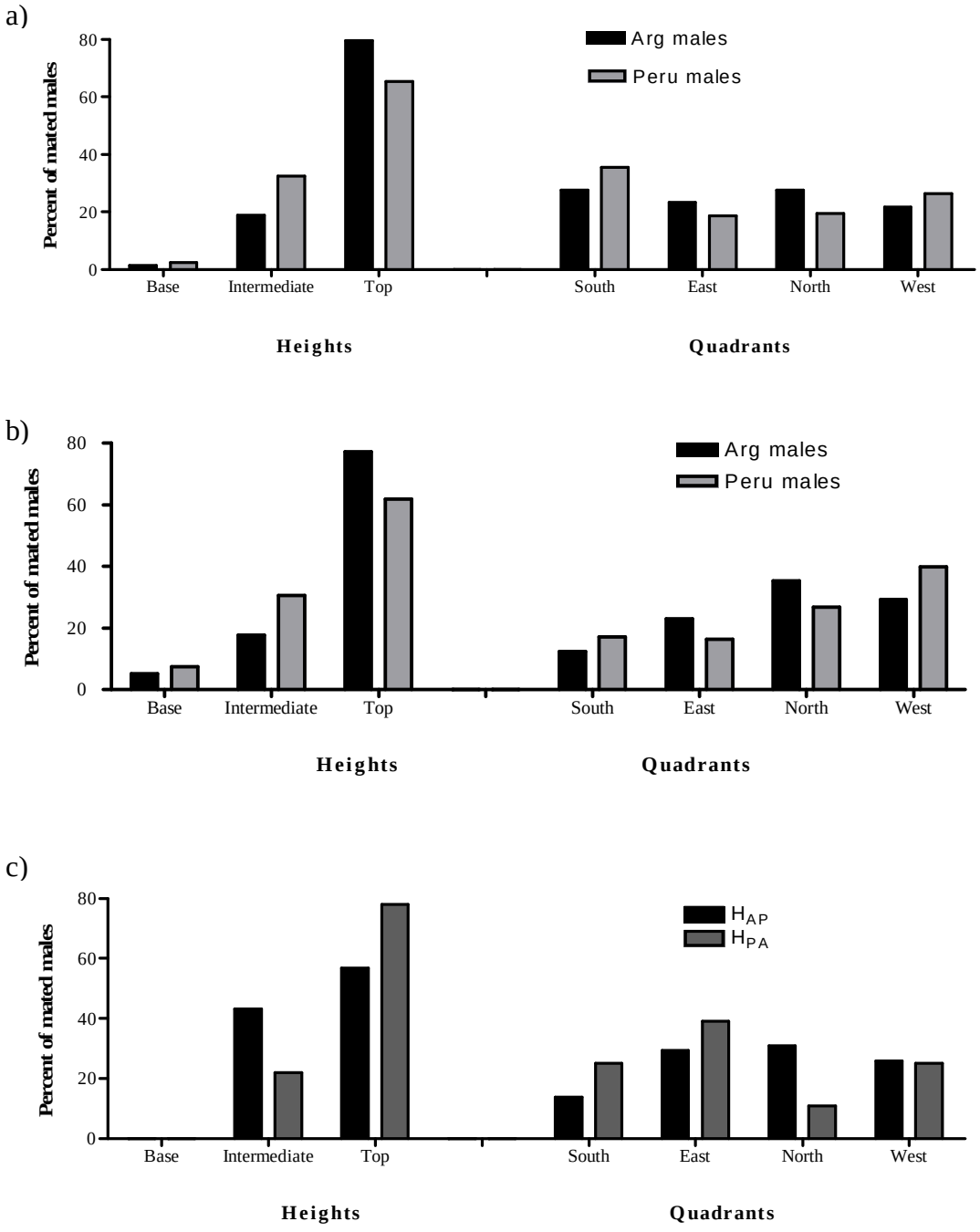

Figure 1. Percent of mating couples of *Anastrepha fraterculus* from Argentina, Peru and hybrids found in the different parts of the tree. a) Bisexual test with both males and females from the two parental strains. b) Unisexual test with the parental strains and females from only one strain. c) Unisexual test with parental and hybrid males and parental females.

Resúmenes de Posters Presentados/Abstracts of Presented Posters

Four New Species of *Anastrepha* (Diptera: Tephritidae)

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The genus *Anastrepha* (Diptera: Tephritidae) is one of the most important pest for Neotropical fruit. The taxonomy of the genus is wide unknown in Colombia where few systematic surveys had been done. Four new species are described and they were found in Colombia from surveys and/or specimens in collections. One of them belongs to *A. robusta* group; other one is so similar to *A. compresa* and *A. canalis*; the third one has the aculeus tip similar to *A. caudata* and the last one has a very thin aculeus, with a long and sinuous tip.

Two New Species of *Doryctobracon* (Hymenoptera: Braconidae: Opiinae) Parasiting Fruit-Infesting *Anastrepha* (Diptera: Tephritidae)

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Insects in the genus *Anastrepha* (Diptera: Tephritidae) are one of the most important pests for Neotropical fruits. The biological control is an important approach to be used in Integrated Pest Magnament Programs. Natural enemies from the Neotropical Region must be found in order to select the better species to be used. Two new species of *Doryctobracon* (Hym., Braconidae, Opiinae) from Brazil and Colombia are described and they were found in surveys looking for natural enemies of fruit flies. The new species from Colombia is similar to *D. zeteki* and *D. crawfordi*, however, they could be differenced in the body and wing color and/or in the propodeum. The species from Brazil is related to *D. auripennis* but they could be diferrenced in the body and wing color.

Estudio Químico y Morfológico de las Glándulas Salivales Abdominales y el Epitelio Pleural de *Toxotrypana curvicauda*

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Las glándulas salivales abdominales y el epitelio pleural de machos de la mosca de la fruta de la papaya *T. curvicauda* fueron estudiados, químicamente y morfológicamente, de hexánicos mediante

cromatografía de gases/espectrometría de masas y microscopía de luz y microscopía electrónica de transmisión, respectivamente. Se identificó la 2-metil-6-vinil pirazina, componente principal de la feromona sexual, en los extractos hexánicos de glándulas salivales abdominales, pero no en el epitelio pleural. Los machos de *T. curvicauda* presentaron dos estructuras globulares esféricas blancas de masa opaca con numerosas traqueolas, localizadas simétricamente en los segmentos abdominales III y IV muy cercanas a la región pleural, que correspondieron a la glándulas salivales alargadas. Se encontraron también células pleurales alargadas con una cutícula transparente muy vascularizada, ubicadas en posición longitudinal a ambos lados del abdomen, en los segmentos III al V. En los estudios de morfología fina de las glándulas salivales abdominales se observaron numerosas vesículas secretoras, aparato de Golgi y retículo endoplásmico rugoso bien desarrollados, mitocondrias, núcleo excéntrico, traqueolas y una membrana celular con gran cantidad de pliegues, todos ellos característicos de células de tejido secretor.

Especies de *Anastrepha* Schiner Identificadas en la Red de Trampeo Contra Moscas Exóticas (Jackson y Multilure) en Campeche, México

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En Campeche solo cuatro especies del complejo *Anastrepha* se consideran como de importancia económica. *A. ludens* se le encuentra asociada principalmente a naranja, mandarina y toronja; *A. obliqua* es más común en mango y frutos del género *Spondia*; *A. striata* se le considera que únicamente ataca a guayaba y *A. serpentina* ataca a una gran variedad de zapotes en los que destaca el mamey y el chicozapote. Otras especies como *A. ampliata, A. spatulata, A. pallens A. chiclayae, A. Sagittata* y *A. Robusta* se han encontrado en cultivos de mango y toronja, mientras que *Anastrepha fraterculus* en almendros y mango. El desarrollo del presente trabajo se llevó a cabo utilizando material biológico obtenido en la red de trampeo que se realiza en todo el estado de Campeche. La identificación de los ejemplares colectados se lleva a cabo en el laboratorio del Comité con ayuda de un esteroscopio y microscopio, agujas de disección y las claves correspondientes a la familia Tephritidae y el género *Anastrepha* Schiner. Mediante claves se identificaron 7 especies de *Anastrepha* Schiner: *Anastrepha pallens* (Coquillett), *A. chiclayae* (Greene), *A. fraterculus* (Wiedemann), *A. spatulata* (Stone), *A. ampliata (*Hernández*), A. robusta* (Greene) y *A. sagittata* (Stone). El presente trabajo se realizó con la finalidad de tener un conocimiento preciso sobre las diferentes especies de *Anastrepha* presentes en el Estado, lo cual ayudará a reconocer la posible introducción de cualquier mosca exótica que represente un riesgo al Agro Campechano. Estos resultados son preliminares ya que se continúa con la colecta e identificación de especímenes.

Determinación de la Gravidez de Hembras de *Anastrepha obliqua* con Base en la Observación Microscópica de Espermatecas

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En México, la Campaña Mosca de la Fruta a través de sus actividades ha logrado el reconocimiento de áreas libres de moscas de la fruta en el norte del país. En el caso de *Anastrepha obliqua* (Mosca del mango), la verificación del grado de madurez de los órganos reproductores de la misma debe de dictaminarse con mayor precisión la cual determinará el despliegue de la realización del control integrado en la detección de un ejemplar silvestre. Considerando lo anterior este estudio se realizó con el objetivo de verificar diferencias morfológicas entre hembras apareadas fértiles y hembras apareadas estériles. En hembras fértiles apareadas con machos fértiles y estériles mantenidas en proteína hidrolizada fue posible determinar la presencia de esperma en la espermateca durante 9 días. En agua destilada las hembras fértiles apareadas con machos fértiles presentaron espermas durante 9 días, no así las hembras que se aparearon con machos estériles que solo presentaron espermas durante 8 días. En alcohol el tiempo para observar espermas fue de tan solo 7 días para ambos tratamientos. En el caso de las hembras mantenidas en seco, los espermas se apreciaron durante 6 días para ambos tratamientos. Aunque no se logro realizar el conteo de espermas, se observó una cantidad mayor en hembras fértiles apareadas con machos fértiles que hembras fértiles apareadas con machos estériles.Se considera que de acuerdo a los resultados obtenidos, los dictámenes no solamente estarán enfocada a la observación de huevos maduros sino que se complementará la identificación al observar la presencia o ausencia de espermas, dictaminando así si la hembra se encuentra copulada o no, dando como resultado a hembras con huevecillos maduros copuladas o hembras con huevecillos sin copular.

Description of the Third Instar Larvae of *Anastrepha leptozona* Hendel (Diptera: Tephritidae)

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Anasterpha leptozona is a species widely distributed throught the Neotropical region. In this work we made a detailed description of the morphology of the third instar larvae for the first time. Studied specimens were collected infesting fruits of *Micropholis mexicana* in the so-called "El Bucaro" neighborhood of Huehuetán, Chiapas, Mexico. Samples were prepared to be observed in scanning electron microscopy (SEM). The antenno-maxillary complex, oral ridge, labium, stomal sensory organ, cephalopharyngeal skeleton, anterior and posterior spiracles and caudal segment are described and illustrated. Mature larvae presented a "ventral sclerite" bellow the pharyngeal sclerite which is characterized by the first time in *Anastrepha* species. The labium has two papilla sensilla which have not been previously described in other mature larvae of the genus.

Reproductive Phenology of Four *Anastrepha* spp. (Diptera: Tephritidae) in the *Fraterculus* Group in Southern Bahia, Brazil

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The phenology of reproduction is one of the key traits of an organism´s life history. However, there is a paucity of information on reproductive phenology of *Anastrepha* under natural conditions. This information is important for the efficiency of fruit fly control programs. We documented the reproductive phenology of four *Anastrepha* species in the *fraterculus* group, *A. fraterculus*, *A. obliqua*, *A. sororcula*, and *A. zenildae* in southern Bahia, Brazil. Our study site was an organic guava orchard of 0.5 ha within a 30 ha farm located in Una, Bahia and surrounded by mature coastal rainforest. The orchard is comprised of 57 trees of *P. guajava* cv Paluma, and the trees in the orchard are interspersed with banana, cacao, cassava and rubber trees. Fruit fly captures were carried out using 10 plastic McPhail traps baited with protein hydrolisate at 5% and serviced on a weekly basis (7-d intervals). The sampling was carried out from March 2005 through March 2007. All captured adult female fruit flies were individually identified and dissected. The oocytes were measured, counted and those longer than 1 mm were considered mature. A total of 10,312 females of *A. fraterculus*, *A. obliqua*, *A. sororcula*, and *A. zenildae*, were captured. We observed peaks in adult population levels preceding and following peaks in guava production from March to May in each year. A total of 102,579 oocytes was counted and measured for the four *Anastrepha* species. Most *A. fraterculus* (54.6%), *A. obliqua* (63.3%), *A. sororcula* (25.9%), and *A. zenildae* (47.9%) females trapped were gravid. Most *A. fraterculus* (41.3%), *A. sororcula* (53.8%) and *A. zenildae* (45.6%) mature flies were found to carry between one and ten oocytes. Most *A. obliqua* females (31.6%) carried from 11 to 20 oocytes. Activity subsided in the winter due to fewer fruit available, higher pluviosity and lower temperatures.

Longevity-Fertility Trade-offs in *Anastrepha ludens*, Across Dietary-Restriction Gradients

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Adult food, based on hydrolyzed enzymatic yeast plus sugar (1:3 ratio) favors fruit fly sexual maturity, acting as a trigger of reproduction. Contrary, the reduction of yeast in the diet delays reproduction and increases longevity. To understand the relationship between longevity and reproduction, as is affected by nutrition, we evaluated 4,800 *Anastrepha ludens* adults, assessing survival in both sexes and female reproduction. These adults were individually exposed to one of 20 diets, combining four Yeast: Sugar (Y:S) ratios (1:3, 1:9, 1:24 and only sugar) plus 4 starvation controls. Data generated during the 3-year study represents approximately 100,000 day-flies of each sex and 750,000 eggs laid by 2,400 females. The fertility and longevity-extending responses were used to create contour maps (X-Y grid) that show the demographic responses (Z-axis) across dietary gradients that range from complete starvation to both ad libitum sugar-only and ad libitum standard diet (3:1 sugar-to-yeast). Our results showed demographic equivalencies along nutritional gradients, differences in the graded responses of males and

females, and egg production costs that are sensitive to the interaction of food amounts and constituents. The fact that maximum longevity and maximum reproduction occur at different nutritional combinations, challenge the development of optimal diets to improve sterile male performance in SIT programs.

(This presentation is part of the paper "Longevity-Fertility Trade-offs in the Tephritid Fruit Fly, *Anastrepha ludens*, across Dietary-restriction Gradients" published in Aging Cell. Vol. 7: 470-477[(2008])

Efecto de Biopelículas a Base de Quitosan sobre el Desarrollo del Huevo y Larva de *Anastrepha ludens* en Frutos de *Mangifera indica* Cv. Ataulfo

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Los mangos que México exporta están sujetos a regulaciones de cuarentena por presencia de plagas y enfermedades. La hidrotermia es el tratamiento recomendado. Una reciente aplicación es el uso de recubrimientos con diferentes polímeros. Trabajos previos han demostrado que el quitosan al ser aplicado como bio-película alarga la vida de anaquel, reduce los microorganismos e inhibe el desarrollo de huevos de *Anastrepha ludens.* El objetivo del presente trabajo fue determinar el efecto del quitosan sobre el desarrollo de huevos y larvas de primer, segundo y tercer estadio de *A. ludens* en frutos de mango cv. Ataulfo. Mangos de tres cuartos de madurez se infestaron durante 4h. Los tratamientos estuvieron determinados por el tiempo en el cual se aplicó el biorrecubrimiento: 5 y 48h para el estadio de huevo y 6, 9 y 11 días para los estadios de larvas de primero, segundo y tercer, respectivamente. Como control se utilizaron frutos infestados sin biorrecubrimiento. Los tratamientos se desarrollaron a 26°C hasta completar el desarrollo larvario. Los resultados mostraron un 100% de mortalidad en huevo de 5 y 48 horas de edad y en larvas de primer estadio. En segundo y tercer estadio larval la mortalidad fue de 94% y 0.07%. La falta de desarrollo de huevos y larvas de primero y segundo estadio pudo haber sido ocasionado por la modificación en la transferencia de masas (oxígeno, agua, etcétera). La ausencia de efecto sobre larvas de tercer estadio se debió, primordialmente, al estado de deterioro del fruto, lo que impidió la formación uniforme de la biopelícula. Las sobrevivientes de segundo estadio presentaron menor porcentaje de pupación ($F=9.65, gl=2,45, P\leq 0.0003$), de voladoras ($F= 78.03$, $P\leq 0.0001$) y de emergencia ($F= 0.85$, $gl=2,45, P\leq 0.04306$) causado, probablemente, por anoxia y no se observó diferencia significativa en peso de larva ($F=1.77, gl=2,45$, $P\leq 0.1806$) y de pupa ($F=0.0053, gl=2,45, P\leq 0.9948$).

Differences in Sexual Maturation among Several Strains of *Anastrepha fraterculus* (Diptera: Tephritidae)

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Sexual maturation is a relatively long process in *Anastrepha* males. This produces an increase in the costs to apply the Sterile Insect Technique (SIT) because the flies must be maintained in laboratory conditions for several days before released. Our objective was to study the duration of sexual maturation process in different mutant strains in *Anastrepha fraterculus* and to study the heredability of this trait. We also analyzed the effect of the juvenile hormone treatment on the sexual maturation on males from some of the mutant strains. To estimate sexual maturity we visually 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 studied five strains: an eye color mutant (#3210), a body color mutant (#3312), two wild-type strains with different inbreeding levels (#2975 and L-TUC) and a wild population (W-TUC). We found that males from strain 3210 have a significantly shorter (than the others) pre-copulatory period. The wild population showed longer times than the two wild-type laboratory strains, which were not different. Topical applications of the juvenile hormone analog, methoprene, to newly emerged males showed an additional reduction in the time needed for #3210 males to reach sexual maturity. These results encourage the use of this strain of *A. fraterculus* as a model to study the inheritance of the sexual maturation and as a potential laboratory strain to develop SIT for this fruit fly.

Exposure to Guava Increases Male Sexual Competitiveness in the South American Fruit Fly, *Anastrepha fraterculus*

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Exposure to plants has been shown to influence sexual behavior in a number of phytophagous insect species. Here we examined the effect of fruit exposure on the mating competitiveness of male *Anastrepha fraterculus*, by evaluating six fruit types, different durations of exposure and the need of physical contact with the fruit. In grapefruit, lemon, and orange males were exposed to essential oils emanating from glands in the flavedo area, whereas mango, papaya and guava were cut in halves so that males had access to the pulp. To run a test ten treated males, ten unexposed control males, and ten females were placed in plexiglass cages and the number of couples obtained for each male type was monitored for two hours. Results showed that exposure to guava increased sexual performance, whereas exposure to lemon and grapefruit decreased it. Different exposures of 1, 5, 10 or 15 days to mango had no effect on male competitiveness, whereas in lemon exposures longer than one day eliminated the detrimental impact on mating competitiveness. The duration of exposure on guavas had also an effect, but this varied between replicates and two categories of males used (wild and laboratory). Mating advantage did not require physical contact with guava and the possible ingestion of fruit compounds, since plain exposure to volatiles ensured the effect on mating competitiveness. The possibility of implementing guavas to enhance *A. fraterculus* male sexual competitiveness in control operations and the potential role of the compound α-copane are discussed.

Impact of Adult Feeding on Fertility, Fecundity and Survival of *Anastrepha fraterculus*, Wiedemann (Diptera: Tephritidae)

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Adult insects can compensate from deficient diets by balancing nutrient intake; however, this is energetically demanding. Aiming at maximizing fitness components of *Anastrepha fraterculus* rearing, we evaluated diets of different nutritional values and delivery schedules. Individual intake was measured daily for two weeks following adult emergence as a direct indicator of food consumption and ability to select and discern nutrients. Five options were evaluated: sugar only, protein only, a combination of sugar and protein (3:1), the same combination with an extra source of sugar, and sugar and protein offered separately. Then, we set up cohort cages with 100 couples. Two food sources were evaluated: sugar or the standard diet (sugar, hydrolyzed yeast, hydrolyzed corn, and vitamins), which were provided either alone or in a combined form in two delivery plates. Based on the diet provided and the period of food exposure we established six treatments. The first set of experiments revealed that, for both sexes, the cumulative sugar intake was higher for flies which were offered sugar and protein in separate plates compared to those were the nutrients were given in combination (same plate). In cohort cages, flies provided with the standard diet and complemented with an additional source of sugar lived longer than control cages. Fecundity was reduced in flies deprived from a protein source from day 0 to 4. Females offered the two food sources on different delivery plates exhibited increased fecundity, twice as much as those of the control cages. Our results suggest that (a) adult physiological needs drive food selection, and (b) providing protein and sugar mixed is detrimental for both fecundity and longevity. We propose that complementing the diet with an additional sugar source may increase production and reduce the amounts of protein used at the rearing facilities.

Characterization of Genetic Variability in *Ceratitis capitata* Strains Using RAPD and Microsatellite Markers

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Flies of the family Tephritidae are mass-reared under laboratory conditions in order to be used in control strategies. This process involves genetic changes due to the adaptation of the individuals to an artificial environment, with lost of phenotypic and behavioural traits related to their performance in the field. The estimation of genetic diversity is considered a powerful tool to measure the genetic potential of strains, and may be useful for monitoring the fitness of insects to guarantee their competitiveness in the field. In the present work we analyzed the genetic variability of two laboratory strains and one wild population of *Ceratitis capitata* (Diptera: Tephritidae). DNA from 10 Individual flies was studied using RAPD and microsatellite markers. The RAPD oligonucleotides AA01, N04, AA3, AM9, AG7, AG12 and O06 were tested, and 20 out of 68

RAPD loci were selected by *Fst* analysis, finding in average 65 % polymorphic loci in the populations. The microsatellite loci ccmic6, ccmic7, ccmic8, ccmic9 and ccmic14 were tested. The number of observed alleles across loci ranged from 2 to 6. Several characteristic alleles were identified in each population. The analysis of the allele frequencies, using both markers, showed significant differentiation ($p<0.05$) between the laboratory strains and the wild population. The AMOVA analysis indicated that the 80% of the variability is found among individuals within each population. This result could be explained because these markers present high level of polymorphism and are no linked to genes involved in the fitness in the field. This work represents the first attempt to study insect quality under laboratory rearing conditions, using molecular markers.

C-Banding and Fluorescence *in situ* Hybridization in *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae)

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In parasitoids of economic importance the cytogenetic characterization may help in a correct identification of the species, improving their application in Biological Control (BC) programs. The better knowledge of the chromosomes' morphology and composition also enables the localization of genes of interest in the karyotype, for instance, those involved in sex determination. Cytogenetic reports in endoparasitoids are relatively scarce because the immature stages of development occur inside the puparium of the host, making it difficult to obtain cells at the appropriate developmental time for chromosome studies. *Diachasmimorpha longicaudata* is a hymenopteran parasitoid widely used in BC strategies against fruit flies, that can be considered a model organism to perform genetic studies. In time, these studies could be extended to other solitary parasitoids whose rearing in artificial conditions is more limited. Our cytogenetic studies in *D. longicaudata* confirmed that females have 2n=40 chromosomes and males n=20 chromosomes (15 metacentrics-submetacentrics and 5 telocentrics). The C-banding pattern consists of pericentric heterochromatic blocks, and reveals the presence of "pseudoacrocentric" chromosomes (acrocentric chromosomes with a completely heterochromatic short arm). Fluorescence *in situ* hybridization with an autologous probe of 18S rDNA revealed 6 nucleolar organizing regions in the haploid complement. These data provide precise cytogenetic information that will help to localize specific genes in the karyotype of *D. longicaudata*.

Characterization of a Genomic Region in *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) Based on Sequences Involved in Sex Determination in Honeybee

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Haplodiploidism, i.e. the presence of haploid males developed from unfertilized eggs and diploid females derived from fertilized eggs, is the rule in Hymenoptera, although, in some species, diploid males have been reported. Different mechanisms have been postulated to explain the sex determination, being the *csd* (complementary sex determiner) system in *Apis mellifera* (Hymenoptera: Apidae) the best characterized. This mechanism is mediated by complementary alleles for a single locus. Under this system females are heterozigous and males are hemizygous (haploid) or homozygous (diploid) for *csd*. The present work describes the characterization of a genomic region in the parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae), based on available DNA sequences and primers from *A. mellifera*. DNAs from individual wasps were purified, and 750 bp long PCR fragments were amplified. PCR products from 27 individuals were purified, sequenced and assembled in a consensus sequence. Conserved nucleotide sequences were observed. Only one A/T base change was found. This base change could be detected digesting the PCR fragment with the restriction enzyme *Mbo*II. Mendelian inheritance of the two sequence variants in the haploid progeny was observed. Preliminary analysis of sex association showed 7 out of 11 heterozygous females. The evaluation of another region in *D. longicaudata* genome, likely to be linked to the first one studied, is in progress. Sex association is expected using the data obtained from both regions. A study of isofemale lines, about the sex ratio, and the inheritance of these genomic regions, has also been started. Our work represents the first approach in the study of sex determination at molecular level in this species widely used as a biological control agent of fruit flies.

Reproductive Isolation among Populations of *Rhagoletis pomonella* in Mexico

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Four ecologically and genetically distinct *R. pomonella* populations that exploit hosts adapted to different environments across a geographical range spanning from South Eastern Canada to Chiapas Mexico have been discovered. Such populations vary in degree of genetic similarity, and although there is evidence of past gene flow, nothing is known about their current reproductive status. We conducted a series of laboratory experiments aimed to determine if parapatric and allopatric populations of *R. pomonella* are reproductively isolated among themselves. Crossing experiments revealed some form of prezygotic reproductive isolation between flies exploiting late fruiting hawthorns in the Mexican Trans Volcanic Belt, parapatric populations in the Sierra Madre Oriental, and allopatric US and Chiapas populations. We also detected different degrees of genetic incompatibility resulting in some combinations unable to produce F1 hybrids, and others unable to produce F2 hybrids (US males and Chiapas females). Such findings might be explained by range contraction and contact of *R. pomonella* populations during early Pleistocene glaciations, followed by expansion and appearance of geographical barriers during the middle Pleistocene.

Random Mating among Allopatric and Ecologically Distinct Populations of *Anastrepha ludens* (Diptera: Tephritidae) in Mexico

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Anastrepha ludens (Loew) (Diptera: Tephritidae), the Mexican Fruit Fly, is a polyphagous pestiferous species with a distribution range encompassing variable environmental conditions. Considering that cryptic species have been found among South American representatives of the same taxonomic group, we tested whether or not *A. ludens* adults have evolved assortative mating as an isolating mechanism that maintains intrapopulation genetic differences and behavioral adaptations to local conditions. Males and females recovered from infested fruit from widely separated locations (Atlantic and Pacific) with similar environmental conditions and males and females stemming from populations within individual-flight range, but collected in different hosts (native and exotic), mated randomly amongst themselves in a field cage. Despite the fact that sibling males and females from two distinct populations also mated randomly amongst themselves, siblings engaged in significantly longer copulations than non-siblings, indicating perhaps that adults discriminated against mates with similar genetic compositions. In view of an ongoing area-wide *A. ludens* eradication campaign in Mexico, our results have important practical implications.

Reproductive Trade-Offs from Mating with a Successful Male: The Case of the Tephritid Fly *Anastrepha obliqua*

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In lekking species, females may become sperm-limited when mating with sexually successful males and this may be exacerbated by a poor male diet. Polygynous males may also be limited by the amount of accessory gland products (AGPs) they can transmit to females, which in turn may influence the females' refractory period and longevity. Here, we tested the effect of male mating history, larval and adult diet on copula duration, mating intervals, female fecundity, fertilisation success, life-span and likelihood to remate using sexually successful males of the lekking tephritid fly *Anastrepha obliqua.* Flies originated from either a native or exotic host fruit and were protein fed or deprived. Male diet and larval host influenced copula duration, while the time elapsed between matings was affected by the interaction of mating order and male adult diet. Female fecundity was not influenced by female position in mating order or protein inclusion into the male diet. However, mating order and male larval diet influenced female fertilisation success. Importantly, as males mated successively they were less able to induce a refractory period on females, as the last females to mate with a male where more likely to remate, and had slightly longer life spans than the first females to mate with males. These results might be attributed to a

decrease in male AGPs with increasing male mating frequency. We discuss the role of conditional expression of male mating frequency with respect to *A. obliqua's* life history, the trade-off that females face when mating with a successful male, the effect of larval diet on adult sexual performance and well as the possibility for sexual conflict to occur due to high male mating rates and fitness costs to females.

Especies Nuevas del Género *Anastrepha* Schiner, (Diptera: Tephritidae) en Ecuador

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Tres nuevas especies de moscas de la fruta registradas en Ecuador, son descritas: *Anastrepha tsachila* encontrada en Alluriquín, cantón Santo Domingo de los Colorados, provincia de Pichincha, atacando a frutos de *Gloeospermun grandifolium* Hekking (Violaceae); *Anastrepha rolliniana* encontrada en la localidad de Palora, provincia de Morona Santiago, atacando a frutos de *Rollinia mucosa* (Jacq.) Baillon (Annonaceae), y *Anastrepha mikuymono*, encontrada en el sector Sacha, provincia de Orellana, atacando a frutos de *Pouteria* sp. (Sapotaceae). *A. tsachila* pertenece al grupo fraterculus, especie de tamaño medio, metanoto y sub escutellum claros, pero en la base del primero la coloración amarillenta es más opaca, ápice del aculeus con dientes estriados. *A. rolliniana*, perteneciente al grupo fraterculus, especie de tamaño grande, se caracteriza por tener las tres bandas unidas, banda en S con una incisión en su base, vena M notoriamente curvada en el ápice. *A. mikuymono*, perteneciente al grupo leptozona, especie de tamaño medio, color amarillento, cabeza con un solo par de setas orbitales superiores, ala con las tres bandas típicas, separadas; vena M sin curvatura en el ápice, brazo interno de la banda en V bien proyectado hacia atrás, pero sin llegar a topar la banda en S.

Sesión/Session III

Demografía y Comportamiento/Demography and Behavior

Biodemografía de la Mosca Mexicana de la Fruta: Avances en el Conocimiento e Implicaciones para los Programas de Manejo

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Se presentan los resultados sobre investigaciones recientes realizadas en condiciones de laboratorio con la mosca Mexicana de la fruta *Anastrepha ludens*. El objetivo fue el de determinar los rasgos biodemográficos característicos de esta especie, su comparación con la mosca del Mediterráneo, así como determinar el papel que juega la nutrición en la fecundidad y sobrevivencia de esta especie.

A partir del seguimiento de un total de 1100 hembras mantenidas en jaulas individuales se determinó la edad a la madurez sexual, las tasas de fecundidad diaria y a lo largo de toda la vida, así como la longevidad.

Nuestros resultados revelan que, con relación a la mosca del Mediterráneo, *Ceratitis capitata*, la mosca Mexicana es una especie más lenta en madurar (14 vs. 17 días), más fecunda (1100 vs 1400 huevos por hembra), y más longeva (35 vs. 50 días).

Estos resultados confirman la generalidad de ciertos descubrimientos hechos con la mosca del Mediterráneo, como la desaceleración de la mortalidad en edades avanzadas, y la débil correlación entre producción de huevos a edad temprana, con la subsecuente reproducción y la longevidad (el “costo de la reproducción”).

Las investigaciones sobre el efecto de la nutrición, confirmaron que la dieta tiene un efecto determinante sobre la fecundidad y sobre la longevidad. Los efectos sobre la longevidad fueron diferentes entre machos y hembras. Las condiciones de alimentación que maximizaron la fecundidad, resultaron diferentes de aquellas que maximizaron la longevidad de las hembras.

Se discutirá sobre las implicaciones que estos conocimientos biodemógráficos pueden tener para los programas operativos de manejo.

Divergence and Distribution of Fruit Flies in the Genus *Rhagoletis* in Mexico

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The genus *Rhagoletis*, was for a long time at the center of a heated debate about the relative importance of different speciation modes. Additionally, the economic importance of some species in the genus has generated numerous studies on the biology and behavior of these flies. Altogether, *Rhagoletis pomonella* is probably the best known Tephritidae species in the word. Despite this fact, little work has been done on the genus outside Chile and South of the U.S.-Mexican border since 1966. Recent work in Mexico has revealed surprising contributions to our knowledge of sympatric speciation events documented and debated in Northeastern U.S. for the past 30 years, reconciling conflicting geographic views about speciation modes. Here we summarize results of work carried out in México over the past seven years in order to establish distribution, divergence, and behavior of flies in the genus *Rhagoletis* and their natural enemies.

Sympatric speciation and the apple maggot fly

Sympatric speciation is the splitting of one evolutionary lineage into two without the occurrence of geographic isolation. In 1860, Benjamin Walsh postulated that the shift of *Rhagoletis pomonella* from native hawthorns to introduced apples was the initial stage of a process leading to speciation. He later proposed that phytophagous insects often speciate through host race formation. The *R. pomonella* case spearheaded a heated debate about the relative importance of different geographic speciation modes (allopatric vs. sympatric) that lasted for half a century. The *R. pomonella* speciation story stood the debate and served to prove that sympatric speciation is possible for phytophagous insects that exhibit host specific mating, host fidelity, and fitness trade-offs in novel and ancestral hosts (see Berlocher & Feder 2002 for a review).

The genus *Rhagoletis* in Mexico: 1966-2003

Research on fruit flies in México focused on the genus *Anastrapha* from the end of the 19th century to 1966 (reviwed in Aluja 2000). In 1966 Bush (1966) reported *R.pomonella* in native hawthorns and apples in central Mexico, and described *Rhagoletis zoqui*, a species associated to walnuts in Hidalgo. Padilla (1964) reported a fly in the genus *Rhagoletis* associated to black cherries in Texcoco which was later identified as *R.* nr *cingulata* by Foote (1981). Hernandez-Ortíz (1985; 1993) later decribed *Rhagoletis turpiniae* associated to *Turpinia insignis* in Veracruz, *Rhagoletis ramosae* associated to walnuts in Guerrero and Michoacan, and *Rhagoletis solanophaga* associated to *Solanum* in Veracruz (Hernandez-Ortíz and Frías 1999). Aluja et al. (2001) reported the only study of a behavioral nature in México for three species in the genus plus a paper touching on distribution of *R. pomonella* and its natural enemies in Veracruz (Aluja et al. 2000).

Allopatric origins of genetic variation allow sympatric speciation.

Just after a number of valid cases of sympatric speciation through host race formation in several insect taxa were made available through research efforts over a couple of decades suggesting that this process may occur more often than it was thought before in nature (reviewed in Drés and

Mallet 2000), a molecular study of *R. pomonella* populations that included individuals from the central Mexican Altiplano, revealed that the genetic variation allowing flies in the *pomonella* group to adapt to hosts of different fruiting phenologies had its origins among allopatric populations of the central Altiplano in México. Diapause variation contained in chromosome inversions could be traced to Mexican populations by means of discovery of a N-S cline evidencing past geneflow from México to North Eastern U.S. (Feder et al. 2003).

A second Mexican population may have served as a conduit for Altiplano inversions reaching the U.S

A second population of *Rhagoletis pomonella* in Mexico, serving as a conduit for chromosome inversions containing diapause variation was discovered and sequenced in 2005 (Feder et al. 2005). Genetic regions contained in inversions were found to be more recalcitrant to introgression than other regions of the genome, suggesting a story of isolation, secondary contact and introgression.

Distribution and divergence of *R. pomonella* in México.

A comprehensive study on distribution, host range and phenology of *R. pomonella* in México (Rull et al. 2006) revealed the existence of three ecologically distinct populations in México. A population exploiting late fruiting hawthorns in the Eje Volcanico Trans Méxicano, ranging from Puebla to Jalisco exhibiting long diapause schedules adapted to the late fruiting phenology of its hosts. A second population exploiting several native species of early fruiting hawthorns on the Mexican Sierra Madre Oriental from Veracruz to Coahuila, and an isolated population in Chiapas exploiting late fruiting hawthorns and exhibiting long diapause. The three populations are genetically distinct, and differ in genetic composition in different degrees from U.S. populations (Xie et al. 2007; Michel et al. 2007). A comprehensive phylogeny of the *pomonella* species group including Mexican populations later confirmed hypotheses about plural speciation mode allowing divergence in the group (Xie et al. 2008).

Cascading differentiation across trophic levels.

A recent unpublished study (Rull et al. unpublished) dealing with distribution and phenology of parasitoids associated to hawthorn infesting *Rhagoletis* in México revealed important differences in parasitoid guild composition and parasitism rates across the whole *R. pomonella* ditributional range. More importantly, some parasitoid species found across the entire fly range (notably *Utetes canaliculatus*) have evolved diapause regimes closely matching those of the different fly populations they exploit. Pending genetic analysis will reveal if differentiation among populations of these species matches the story unraveled for their hosts.

Evolution of reproductive isolation among Mexican and U.S. populations.

Unpublished crossing experiments (Rull et al. unpublished) have revealed some degree of reproductive incompatibility among EVTM flies and other populations, and between Chiapas and U.S populations.

Current studies are underway to establish the range and degree of ecological divergence among Black Cherry and Walnut infesting flies in the genus *Rhagoletis* in México, such studies will hopefully allow to widen our understanding of the evolutionary history of the whole group and

complemented with genetic studies and studies of reproductive compatibility will allow us to better understand speciation events in phytphagous specialist insect groups.

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Resúmenes de Posters Presentados/Abstracts of Presented Posters

Diurnal Resource Use in a Tree Canopy by Wild, and Fertile and Sterile Mass-Reared Queensland Fruit Flies, *Bactrocera tryoni*

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Sterile insect technique (SIT) is a cornerstone for area-wide control of Queensland fruit flies (Q-flies) in Australia. Effective SIT programs are entirely dependent on the ability of released flies to (1) survive in the field and (2) find and mate with wild flies. However, the mass-rearing environment used to produce the millions of flies required each week for SIT may produce sterile flies that are not adequately capable of either key task. Surprisingly, to date, there has been no direct measurement of the ability of sterile Q-flies to adjust to field conditions. We assessed the diurnal spatial distribution and microclimates favored by wild, fertile mass-reared, and sterile mass-reared Q-flies, and their survival, in potted citrus trees within field cages. Feeding behavior of wild, and mass-reared fertile and sterile Q-flies was strongly associated with the upper surface of leaves. Height of all Q-fly types varied during the day. Mean height was highest at dusk, and lowest at midday (the hottest part of the day), but mean height of sterile flies throughout the day varied less than that of wild flies. Mass-reared flies were found in locations that were cooler and darker than those used by wild flies at midday. Survival of fertile and sterile mass-reared flies was lower than that of wild Q-flies. Our results to date suggest that physical microenvironment of sterile Q-flies differs from that of wild flies, and that this could contribute to poor performance in the field following release.

Efecto del Fruto Hospedero y Coespecíficos sobre la Liberación de Feromona Sexual en *Toxotrypana curvicauda*

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En este trabajo se estudió el efecto de la edad, fruto hospedero, coespecíficos y fruto con coespecíficos, sobre la liberación de la feromona sexual de *T. curvicauda*. Se formaron 6 grupos de machos. En un grupo (control) los individuos se mantuvieron en aislamiento visual y olfativo durante el tiempo en que se realizaron las mediciones para evaluar el efecto de la edad; en los siguientes grupos cada macho fue colocado durante 1 h en un recipiente de acrílico, antes de capturar con fibra SPME los volátiles emitidos, bajo una de las siguientes condiciones: con un fruto hospedero (F), 2 hembras (2H), 2 machos (2M), 2 hembras + fruto (2H+F) ó 2 machos + fruto (2M+F). En todos los grupos, se capturó la 2,6-metilvinilpirazina (2,6-mvp) durante 3 h, los 13 días posteriores a la emergencia de los machos. La 2,6-mvp se identificó y cuantificó con un

cromatógrafo de gases acoplado a espectrómetro de masas. Todos los grupos presentaron un mismo patrón temporal de liberación los primeros 5 días. La liberación máxima de 2,6-mvp en el grupo control se presentó el día 7, mientras que en los grupos F, 2H, 2M, 2H+F y 2M+F se presentó los días 13, 11, 5, 3 y 3, respectivamente. Sólo los grupos 2H y 2H+F liberaron una cantidad significativa mayor de 2,6-mvp que el control. Se encontró una interacción significativa entre el tratamiento y la edad de los machos en los grupos F, 2H y 2M.

Influencia de la Edad, Hora del Día y Tipo de Planta en el Comportamiento de *Toxotrypana curvicauda*

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La mosca de la fruta de la papaya, *Toxotrypana curvicauda*, es una especie que se encuentra distribuida en áreas tropicales y subtropicales de América y está asociada a plantas de las familias Caricaceae y Asclepiadaceae. El comportamiento de *T. curvicauda* asociado a su principal hospedero (*Carica papaya*) ha sido estudiado por varios autores. Sin embargo, ningún estudio ha hecho un seguimiento individualizado de las actividades diarias y la distribución espacial de esta mosca y no se conocen las características de los sitios de refugio utilizados por esta especie. En una jaula de campo donde se encontraba una planta de *C. papaya* y dos plantas no hospedera (*Schefflera actinophylla* y *Ficus benjamina*) se liberaron 10 machos y 10 hembras recién emergidos, registrándose sus actividades diariamente durante 10 días consecutivos, a partir de las 9:00 hasta las 17:00. Se encontró que moscas recién emergidas visitan la planta hospedera, aunque permanecen poco tiempo en ella. La permanencia de hembras aumenta significativamente hasta después de 6 d de edad. Los insectos se desplazaron durante la noche a las plantas no hospederas y se dirigieron a la planta hospedera durante el día. Los machos pasan casi el doble de tiempo en el hospedero que las hembras. Los machos visitaron más veces los frutos que las hembras donde se observó más del 80% de los llamados. Toda la actividad sexual se observó en la planta hospedera. No se observó preferencia de hembras o machos hacia ninguna de las plantas no hospederas.

Selección Sexual de *Toxotrypana curvicauda* en Relación a su Edad, Virginidad y Peso

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La mosca de la fruta de la papaya**,** *Toxotrypana curvicauda* Gerstaecker, es una plaga de la papaya, *Carica papaya* L que se distribuye en las zonas tropicales y subtropicales de América. Debido a su importancia económica, se ha estudiado la biología de este insecto por varios autores en *C. papaya*. Hasta la fecha no se ha estudiado el proceso de selección de pareja entre individuos

de esta especie. El estudio del sistema de apareamiento de un insecto permite conocer los atributos que son seleccionados y como esta selección cambia con el tiempo. Es posible, además correlacionar ciertas decisiones de los individuos con su fecundidad y por ende, con el crecimiento poblacional y la aparición de alguna plaga. Se evalúo la influencia de la edad, virginidad y tamaño en el proceso de selección de pareja de *T. curvicauda*. Se consideraron insectos jóvenes (6-8 d de edad) y viejos (11-13 d de edad), insectos pesados y ligeros y vírgenes y no vírgenes. Los resultados muestran que ni el peso ni la edad de los insectos son factores de discriminación por parte de hembras o machos. Sin embargo, los machos seleccionaron significativamente hembras vírgenes para el apareamiento.

Llamado Sexual de *Anastrepha serpentina* (Wiedemann) (Diptera: Tephritidae)

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El comportamiento de llamado de machos de laboratorio y silvestres de *A. serpentina* fue estudiado en laboratorio y jaulas de campo. En el laboratorio, ambas cepas iniciaron el llamado entre los 5 y los 7 días de edad. El patrón diario de llamado también fue similar en ambas cepas, iniciando a partir de las 7:30 h, alcanzando el pico máximo de las 14:30 a las 16:30 h y finalizando a las 18:30 h. Los machos silvestres que fueron colocados individualmente en el recipiente (tratamientos de 1, 5 y 10 machos por recipiente en ambas cepas), no llamaron. En las jaulas de campo las diferencias fueron más marcadas. Los machos de laboratorio iniciaron el llamado a los 5 días de edad y alcanzaron el pico máximo de los 8 a los 10 días a una edad más temprana que los machos silvestres (a los 10 días y de los 15 a los 18 días, respectivamente). Otra diferencia observada fue la formación de "leks". Mientras que los machos de laboratorio se agregaron para el llamado solamente durante los dos días de máximo llamado (a los 8 y 9 días de dad), los machos silvestres se agruparon durante todos los días del experimento. La diferencia más notable fue que a diferencia de los resultados obtenidos en el laboratorio donde solamente se observó un pico de llamado en ambas cepas, en las jaulas de campo se observaron dos picos de llamado, uno en la mañana y otro en la tarde, tanto en machos de laboratorio como en los silvestres. Se discuten los resultados en base a la importancia de conocer tanto los efectos de la cría masiva en los machos de *A. serpentina* para el uso eficiente en la TIE, como el comportamiento de esta mosca para la identificación de la feromona sexual.

Evaluación de Diferentes Espectros Lumínicos Sobre el Comportamiento Sexual de *Anastrepha ludens*, Bajo Condiciones de Jaula de Campo

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Bajo condiciones de jaula de campo, se determinó el efecto que ejerce el espectro lumínico de crianza sobre la competencia sexual, latencia y tiempo de cópula de machos y hembras de *A. ludens* de origen de laboratorio. Los tratamientos consistieron en someter a los insectos, desde el momento de su emergencia hasta su madurez sexual (14 ± 2 días), a cuatro diferentes ambientes lumínicos: Luz azul (8883 ± 1974 ^{0}K), luz blanca o de espectro total (4745 ± 25.4 ^{0}K), luz roja (1613 ± 315.7) y en completa oscuridad (0 ^{0}K). Los resultados obtenidos muestran que los machos y hembras de que emergieron, y maduraron sexualmente en condiciones de completa oscuridad, cópularon significativamente en menores proporciones que aquellos insectos que se desarrollaron bajo los otros regímenes lumínicos evaluados. No se observaron diferencias estadísticas en la latencia y el tiempo de cópula entre los insectos expuestos a los diferentes tratamientos.

Pattern of Host Use by Two Competing Fruit Fly (Diptera: Tephritidae) Species

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Sharing limiting resources has got a negative effect on individuals. This can lead to the development of mechanisms that allow them to escape competition. The use of host marking pheromones is an example of these mechanisms, and previous studies in our laboratory showed that such mechanism works intraspecifically and also interespecifically between *Ceratitis capitata* (Cc) and *Anastrepha fraterculus* (Af). We analyze here infestation patterns in guava in 5 sampling sites of Argentina, aiming at determine the relative abundance of these species and whether they tend to avoid interspecific competition in the field following what was found in laboratory conditions. Fortnightly samplings were made during the whole fruiting season. Fruits were kept individually and the number of pupae of each species was recorded. A relative abundance index was calculated using the number of recovered pupae (RAI = Cc/(Cc+Af)). We found that Af predominated over Cc in almost every tree, although the relative importance of Cc increased along the season. Strong variation in RAI within sites showed there is a strong local effect on host utilization, indicating differences with previous reports. RAI patterns were opposite to those expected according to the degree of environmental disturb. Several cases of fruit simultaneously infested by both species were found along the season. This shows that competition avoidance mechanisms described in laboratory did not prevent interespecific competition. Observed frequencies of co-infested fruit were compared with the expected values under three models of host use (random oviposition and two models that consider different avoidance rates). The random oviposition model was generally the best model, fitting the data in almost every tree. The other two models fitted the co-infestation pattern in only 50-70% of the cases, with some trees fitting better than others. Possible causes of this variation are discussed.

Fruit Fly Larval Monitoring in the Fruit Growing Area of Livramento de Nossa Senhora, Bahia, Brazil.

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Larval monitoring is an important tool for fruit fly detection, to establish the infestation level and the relation between host and fruit fly species. From June 2006 to December 2007, 164 trees of 30 fruit host species and 10 cultivars of mango found in Livramento de Nossa Senhora, a major mango growing and export area. Fruits were collected in the field according their availability and were brought to the laboratory for analysis and rearing the pupa. A total of 23,440 fruits with a total weight of 2,630.89 kilograms were collected. The infestation level ranged from 0.1 to 555.1 pupa/kilo. The highest infestation were found in red mombin, Surinam cherry, yellow mombin, umbu (*Spondias)* and orange. Both, *Anastrepha* and medly infestation was found in the samples. From orange, papaya and Antilles cherry, only medfly was recovered. Non-commercial mangos varieties as 'rosa' and 'espada' were more infested than the commercial ones as Tommy-Atkins, Palmer and Keith. The non-commercial fruits in the gardens and backyards found in the region are primary host of fruit flies and have heavy infestation. Such hosts should be considered as important repository of fruit flies and hence control measures to decrease the adult population have to be applied in order to avoid fruit fly migration into commercial mango orchards.

Remating Inhibition in *Anastrepha serpentina* (Diptera: Tephritidae)

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Chapote (*Manilkara zapota*) a major crop of Mexico, is infested by *Anastrepha serpentina*, which limits its exportation and commercialization. Of the 4 fruit fly species of economic importance for Mexico we have the least amount of information on the biology of *A. serpentina*. In order to implement the sterile insect technique (SIT) for this tephritid, we must first have a better understanding of its sexual behaviour, and specifically on the likelihood of female remating. Sperm and or products of the accessory glands transmitted by males in the ejaculate have been known to be responsible from inhibiting female sexual receptivity. Thus, as a first step towards understanding the mechanism by which female sexual inhibition occurs in *A. serpentina*, we will study if sperm storage by females is correlated to a females' sexual refractory period (time during which females' are not receptive to remate). This project proposes: first, to study the length of the sexual refractory period between the first and second mating of a female; second to study if remating inhibition is related to sperm storage in females; third if sterile males are just as likely to inhibit female remating than fertile mass-reared males and wild males; and fourth to study if the age of sterile males is related to their ability to inhibit female remating in *A. serpentina*. We expect *A. serpentina* to have at least a one-week refractory period comparable to other *Anastrepha* spp. and differing from the relatively short refractory periods of other *Rhagoletis*,

Bactrocera or *Ceratitis* spp. This is the first study to address remating inhibition in *A. serpentina* and the mechanism by which this inhibition takes place in *Anastrepha*. Results from this study will help to improve SIT for this tephritid. This project is currently in progress. Financial support is provided by CONACyT Mexico.

Sesión/Session IV

Dinámica de Poblaciones, Sistemas de Trampeo, Semioquímicos y Hormonas/Population Dynamics, Trapping Systems, Semiochemicals and Hormones

Abiotic Factors and Population Dynamics of Fruit Fly Pests (Diptera: Tephritidae)

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Introduction

Fruit flies are poikilotherms and hence their development and survival is strongly influenced by ambient temperatures. They are highly susceptible to freezing temperatures and thus require the capacity to diapause in order to survive winters in temperate regions. Species in the genus *Rhagoletis,* including the apple maggot and cherry maggot, are examples of fruit flies with this inherent capacity. Fruit flies in the genus *Anastrepha* do not diapause and are thus restricted to tropical and subtropical regions (Flitters and Messenger 1965). High temperatures are also an important mortality factor, especially to the immature stages (Machado *et al*. 1995, McPhail and Bliss 1933). Fruits exposed to direct sunlight can reach temperatures lethal to the larvae (Sivinski *et al*. 2007). The developmental rate of the immature stages and reproductive maturation of the adults are both strongly influenced by temperature. Leyva (1988) calculated the thermal units necessary for the development of *Anastrepha ludens* expressed in degree-days. The formula for degree-days is mathematically simple:

$$^{o}D = [½ (T\ max + T\ min)] – T\ basal$$

The basal temperature is the temperature below which there is no development. For *Anastrepha ludens,* and the other few species tested, the basal temperature is around 9-10ºC (Table 1).

Table 1. Degree-Days for development of some fruit fly species.

	Anastrepha ludens	*Ceratitis capitata*	*Batrocera orientalis*
T basal	9.4 ºC	10ºC	10ºC
Larva	290	200	150
Pupa	315	155	195
Adult	120	70	130
One life cycle	725	435	485

In *A. ludens* the larval and pupal stages each require around 300 degree-days for a total of 600 degree-days to develop from an egg to an adult. Because the female requires another 125 degree-days to become reproductively mature, one complete generation requires approx 725 degree-days. The Medfly by comparison requires only 430 degree-days to complete its life cycle. However, the degree-day model does not always provide a precise estimate of generation time. In A. *ludens* it has been shown that the larvae often do not egress the fruit at the end of development (Thomas 1997). Rather, egression is triggered by some environmental factor, likely related to humidity. Furthermore, the adults are less active when conditions are less favorable (hot, dry weather), and

oviposition cannot occur if fruit are not available. For these reasons, a calendar method is useful for predicting generation time based on prevailing ambientconditions as derived from historical weather records for a particular locality to estimate degree-day accumulations (Table 2).

Table 2. A generation time calendar for *Anastrepha ludens* in the Rio Grande Valley of Texas. Beginning with a fly find from any Julian week, the beginning of the next generation is provided under the Days column, and the end of the quarantine period based on two life cycles is shown in the Q-Weeks column.

	JULIAN WEEK	DAYS	Q-WEEKS		JULIAN WEEK	DAYS	Q-WEEKS
1	(Jan 1-7)	91	21	27	(Jul 2-8)	39	12
2	(Jan 8-14)	86	20	28	(Jul 9-15)	39	13
3	(Jan 15-21)	82	19	29	(Jul 16-22)	39	13
4	(Jan 22-28)	77	18	30	(Jul 23-29)	39	13
5	(Jan 29-4)	73	18	31	(Jul 30-5)	39	14
6	(Feb 5-11)	70	17	32	(Aug 6-12)	40	14
7	(Feb 12-18)	66	16	33	(Aug 13-19)	41	15
8	(Feb 19-25)	62	15	34	(Aug 20-26)	43	17
9	(Feb 26-4)	59	15	35	(Aug 27-2)	44	18
10	(Mar 5-11)	58	15	36	(Sep 3-9)	45	20
11	(Mar 12-18)	55	14	37	(Sep 10-16)	47	22
12	(Mar 19-25	52	14	38	(Sep 17-23)	50	23
13	(Mar 26-1	50	14	39	(Sep 24-30)	55	24
14	(Apr 2-8)	49	14	40	(Oct 1-7)	58	24
15	(Apr 9-15)	47	13	41	(Oct 8-14)	67	24
16	(Apr 16-22)	46	13	42	(Oct 15-21)	75	24
17	(Apr 23-29)	45	13	43	(Oct 22-28)	86	24
18	(Apr 30-6)	44	13	44	(Oct 29-4)	97	24
19	(May 7-13)	43	13	45	(Nov 4-11)	101	24
20	(May 14-20)	42	13	46	(Nov 12-18)	104	24
21	(May 21-27)	41	12	47	(Nov 19-25)	105	24
22	(May 28-3)	40	11	48	(Nov 26-2)	105	23
23	(Jun 4-10)	40	11	49	(Dec 3-9)	103	23
24	(Jun 11-17)	40	11	50	(Dec 10-16)	101	22
25	(Jun 18-24)	40	12	51	(Dec 17-23)	98	22
26	(Jun 25-1)	39	12	52	(Dec 23-31	95	21

Precipitation also exerts a strong impact on fruit fly populations although the effect is often indirect by its influence on fruit phenology, the most critical factor determining the life cycle of fruit flies (Celedonio *et al*. 1995). For example, the native host for *A. ludens* in Mexico is the yellow chapote which grows along the margins of streams in the Sierra Madre Oriental. The yellow chapote typically produces fruit in the springtime and the peaks in Mexfly populations naturally coincide with fruition. Thus, when the chapote crop is early, the Mexfly population also peaks early. Fruit phenology depends on ambient conditions, in particular temperature and rainfall, of which the latter tends to be the more variable. The peak in fruit production, and thus the peak in fly populations, can occur in the spring or the early summer, depending largely on the abundance and timing of rainfall (Thomas 2003). Because the larval stages are adapted to what is essentially a liquid habitat inside the fruit, heavy rainfall is rarely a mortality factor. The larval stages can survive four days completely immersed underwater before measurable mortality is induced.

Wind is an important factor in fruit fly biology. Winds can carry the adults much greater distances than they are capable of dispersing under their own flight power (Thomas and Loera

1998). Female flies are attracted to fruit odors and to the sex pheromones of the males. Because these odors are carried by air movement, the female search flights are oriented to the wind (Robacker 1988).

Sunlight also influences the behavior of fruit flies. The adults are not active at night and do not fly in darkness (Flitters 1964). It is for this reason that sterile releases are only made by day. Sexual activity is strongly influenced by photoperiod (Robacker *et al*. 1991, Epsky and Heath 1993). For example, among the cryptic species related to *Anastrepha fraterculus* some mate at dusk, while others mate only in the morning. Experiments with the Mexfly demonstrated that shifting the photoperiod caused a corresponding shift in sexual activity. Clearly, knowledge of mating behavior is critical to the effective application of SIT programs.

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The Geographic Information System in the Medfly Regional Program Mexico-Belize-Guatemala-USA

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GIS: Main Concepts

A Geographic Information System (GIS) is a computerized system to acquire, store, analyze and display geographic data which is used to support the decision making process. In a GIS environment different phenomena of the real world are represented as thematic layers. Each layer contains the location and the characteristics of the phenomena, and are stored in the computer as vectors (coordinates representing points -pair of coordinates- , lines -a connected set of points- or polygons -line closed at the beginning-) or raster (a continued net of quadrangular features).

The key issue of a GIS is that, with the adequate information stored, complex spatial analyses are possible with the geographic data (location) and its attributes. If the phenomenon of interest is dynamic on time, as the behavior of the medfly populations, and the information is stored on a periodical basis, it is also possible to make temporal analysis. In the case of the Medfly GIS, the basic information comes from two sources: the traps installed in the field and the fruit sampling. For each trap and each place that is sampled, a pair of coordinates with a GPS receiver is registered. The location and other relevant information (trap type, host, medfly sterile and wild captures, and number of larvae) are stored in a database. With the location, the traps and its information are represented as a point layer in the GIS. From this layer, different layers to describe the "field scenarios" are generated according to the needs of the program: wild flies, sterile flies, larvae. The overlay of these layers with other thematic information allows different analyses to describe and explain the medfly behavior.

Data Flow on the Medfly Program

The data flow within the Medfly Program is complex. Around 35,000 traps are installed in the entire region (Figure 1 shows the distribution of the traps); from these installed traps approximately 25,000 are serviced each week. Also, on a weekly basis, around 30 Sterile Insect Technique Airplane Blocks are designed and flight for releases on approximately 24 flights (4 diaries). Figure 2 shows the location of the SIT Blocks for week 37 of 2008. The paths of the releases are register by a GPS and stored on a digital file. All the information (trapping, sampling, Aerial SIT releases, Aerial Spray activities) in a regional context (México, Guatemala and Belize) is managed with a GIS: the Medfly GIS. And it has to flow from the field (trapping, sampling, airplanes), to the laboratories, to informatics and then send it by email to the GIS Specialist.

Medfly GIS Database: Trapping, Sampling, SIT Blocks and Thematic information

Most of the information is managed on Personal MS Access Databases, designed on a standardized way, but customized for the needs of each center. There are six Operation Centers in México, four in Guatemala and one in Belize; each center with a Trapping Database installed (Figure 3 shows the locations of these operation centers). Weekly, each center reports the results on tables, which feed

the Regional GIS Database. The requested trapping information to each center is: The unique regional code for each trap, coordinates (X, Y, Z), host where the trap is installed, type of trap, days of exposition, and the sterile and fertile captures on the field.

The SIT Blocks (aerial releases) are stored as polygon registering information regarding the area and the density of release. The paths of the flights are stored as points with the altitude over the sea level and the speed of the airplane flight. To conduct the different analysis required by the Program Directors a thematic dataset has been compiled: land use (mainly coffee, which occurs to be the main host of the medfly in the region), altitude, temperature, political boundaries, location of operation centers, quadrants, water bodies, roads, rivers.

Regular Reports using GIS

Basically, six maps for the region are prepared and published on an Internet site on JPEG format:

1. **Sterile recapture on Jackson Traps**. This map shows the sterile recapture per trap in Flies per Day, using a simbology which classifies the traps in three categories: without captures, higher than zero but less than three flies per day, more than three flies per day.
2. **Fertile captures on the last three weeks.** This map shows the traps with fertile captures using four data frames: one for each week, and one with the 3 weeks together. Also a graph showing the total captures per week is shown.
3. **Fertile captures by week for the year.** A map with the location of all the traps with captures during the year and a graph that shows the total captures per week during the year.
4. **Larval captures on the last three weeks**. This map shows the places with larvae captures using four data frames: one for each week, and one with the 3 weeks together. Also a graph showing the total larvae per week is shown.
5. **Larval captures by week for the year.** A map with the location of all the places with larvae during the year and a graph that shows the total larvae per week during the year.
6. **Active captures**. A map with the location of the active captures (adults and larvae). The "active captures" are selected based on the time required to complete one (for one male) or three life cycles (for larvae, one female, or two or more flies are captured). This means that to the week when the captures are done, the number of weeks required to complete one or three life cycles is added to estimate if the captures are active or not in the current week.

Let the analyses begin!

With the weekly information complex analyses are conducted. Five of the several analyses produced within the program are described here.

Temporal interpolation for the Southwest Area. Using the Inverse Distance Weighted interpolation method and considering all the traps serviced on the week a raster layer with the estimation of the captures is generated. For each year, 52 maps are generated (one per week). The 52 maps are exported to a JPEG format, and with them an animation on GIF format is generated. The animation shows the behavior of the captures during the year. On a several years animation (in this case from 2006 to 2008), the behavior during the time can be illustrated. This animation has shown that the captures, year by year, have four moments: first, the captures are concentrated in the highlands with coffee (with low numbers of captures); second, the captures within the coffee increase and also all the coffee production areas reports captures (highlands and midlands); third, the number of fertile captures are very high within the coffee and there are captures everywhere (not only in the coffee-belt); and four, the captures decrease and comeback to the first moment scenario. In general terms, for the southwest region, the first moment occurs from August to October, the

second moment from November to January, the third moment from February to April, the fourth moment from May to July.

Historical captures by sub-regions. The behavior of the medfly is different within the whole region regarding the number of captures and the time of the year in which this number reaches its peak. Then the Medfly Region has been divided in sub-regions and historical map with captures since 2004 is generated on a monthly basis. The sub-regions have been created considering similarities on environmental conditions and plague behavior.

Medfly and Coffee Phenology in Guatemala and Mexico. A GIS analysis supported the idea that was described by Midgarden and Lira (2006). Here it is presented the abstract of that idea: **"**Coffee is the main host of Medfly (*Ceratitis capitata*) in Mexico and Guatemala; however their host-insect relationship is not well understood. We show that the coffee belt on the slope of the Sierra Madre Mountains demonstrates a gradient in the time of maturation and harvest. Medflies can infest the coffee fruit at low elevations (~400m) in July, and follow the maturing coffee to final harvest at high elevations (~ 2100m) in December. Generation times at the extremes vary from 3 weeks to 3 months due to temperature differences, but the total degree-day accumulation allows for 5-6 generations of unlimited exponential population increase. Pupation times after coffee harvest in December can exceed 90 days, resulting in the emergence of the highest population of adult flies in March-April. Phenologically, the coffee is in its stress phase at this date and coffee berries are scarce, likely resulting in dispersal of mature adults to search for hosts. Many dispersing flies are captured in detection traps in March through April and can be seen as part of an ecological "shell game": the Medfly population outbreaks appear in one location in April (uninfested or host-poor areas west of the leading edge), while the growing population was actually present at another location months earlier (e.g., December in untreated coffee areas to the East). All the maps (which shown the spatial and temporal relationships between the coffee phenology and the medfly) were generated by the GIS. The following layers were produced: estimation of the availability of the coffee by altitude by month, estimated required weeks to complete the medfly life cycle depending on the temperature, monthly captures and the different combination of these layers.

SIT Airplanes Performance Evaluation. To evaluate the performance of the Aerial SIT activities four main parameters are considered: Altitude over the terrain, speed of the airplanes, the percentage of the Jackson traps within the block that reported at least one capture (positive traps) and the Sterile FTD within the block. The GPS' records of the airplanes contain the speed and the altitude over the sea level. Then a GIS analysis is used to estimate the altitude of the airplane over the ground by subtracting the altitude of the terrain (provided by a digital elevation model) from the altitude reported on the GPS' records. The trapping system is used to summarize the Positive Traps and the Sterile FTD within the release blocks. At the end for each SIT release block the following values are calculated on a weekly basis: 1) averaged altitude of the release flights over the ground, 2) averaged speed of the release flights, 3) averaged Sterile FTD and 4) % of positive traps. Figure 4 presents a 3D view of one particular flight.

Selection of Areas for Spraying. The Southwest region is the more problematic area for the program. Because of the limited resources, prioritization to decide which areas for spraying has to be done. A combined criterion has been established to select the spraying areas for this year: Areas with a Fertile FTD of 1.0 or more in three or more weeks. This criterion is intended to combine infested areas and persistence of the plague. To obtain the areas that match this criterion, weekly interpolation of the FTD are generated. Then for each week, a reclassification to a binary scale is made using the criteria of: 1 if the FTD is 1.0 or more, 0 if not; then the weekly reclassified layers are added. From the results of the addition of all the weekly layers a new reclassification is made,

selecting all the areas that have a value of three or more, which means that, at least, on three weeks of the period of interest those areas where infested.

GIS and the decision making process. Each of the analyses described above had an impact on the decision making process, basically by supporting this process. On table 1 a summary of this relationship is presented. Dynamic decisions have been done with dynamic GIS analyses. For each question made by a decision maker, a map is generated to answer that question. The advantage of using a GIS is the efficiency to generate the spatial and temporal analyses to support decisions.

Table 1. GIS analyses made in the Medfly Program and the decisions they supported

#	Analysis	Decision supported
1	Temporal interpolation for the Southwest Area	This analysis allowed a better understanding about the temporal behavior of the medfly, from which derived a plan to increase the control activities when the medfly populations are building up (second moment), and not when they are in the peak and dispersing (third moment).
2	Medfly and Coffee Phenology in Guatemala and Mexico	Related with the previous analysis, also a better understanding of the temporal and spatial behavior of the medfly was allowed, and based on this idea a Gradual Advance Plan was proposed and executed in the first phase, with the idea of spraying in the second moment, and then use SIT blocks to control the remaining populations.
3	Historical captures by sub-regions	Early awareness on when (moment of the year) and where (sub-region) to allocate the resources. This analysis supported the decision of making a dynamic production of sterile flies according to the needs: during some periods of the year less sterile flies are produced, while during other periods more flies are produced.
4	SIT Airplanes Performance Evaluation	The provider was asked to fly according to the parameters required by the Medfly Program of altitude and speed. The GIS analyses allow constant supervision on this issue.
5	Selection of Areas for Spraying	The decision on when and where to spray was made, considering a limited budget for this activity.

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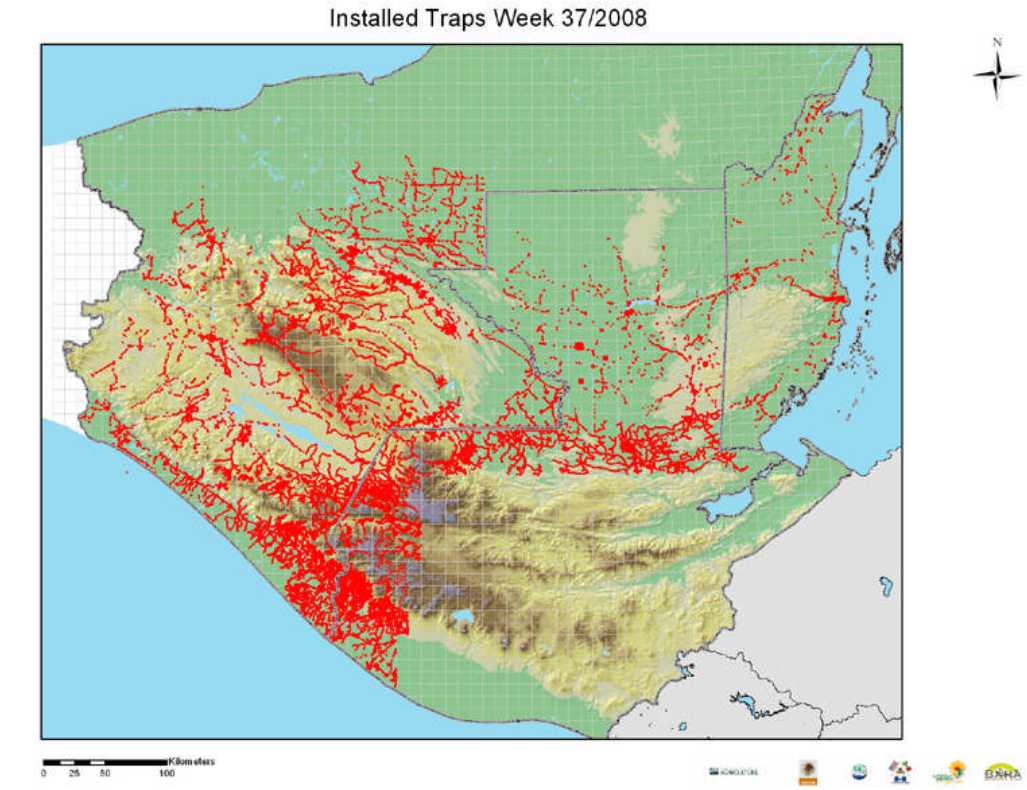

Figure 1. Installed traps on the Medfly Program (Mexico, Guatemala and Belize)

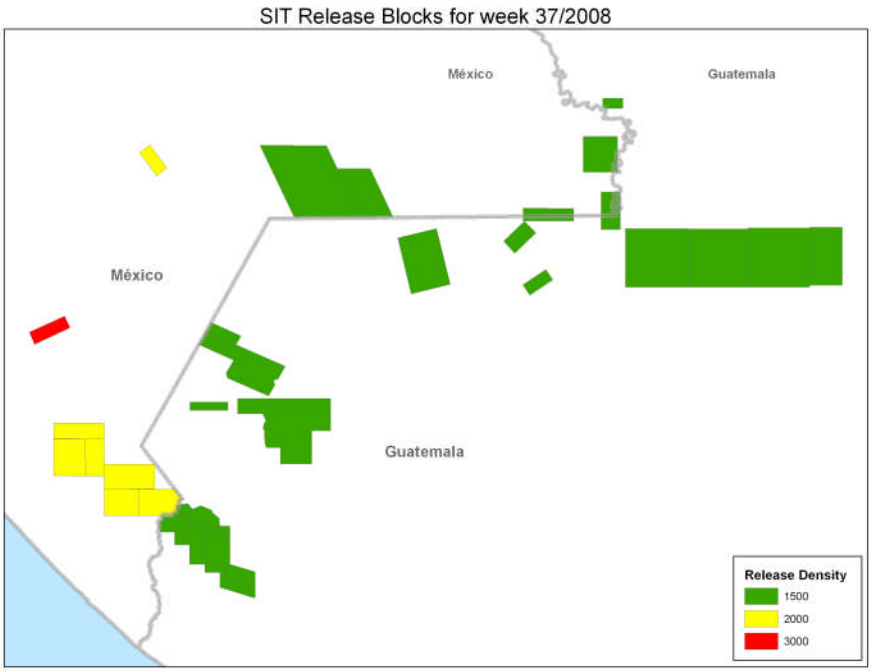

Figure 2. Sterile Insect Technique Release Blocks and its densities for the week 37 of 2008

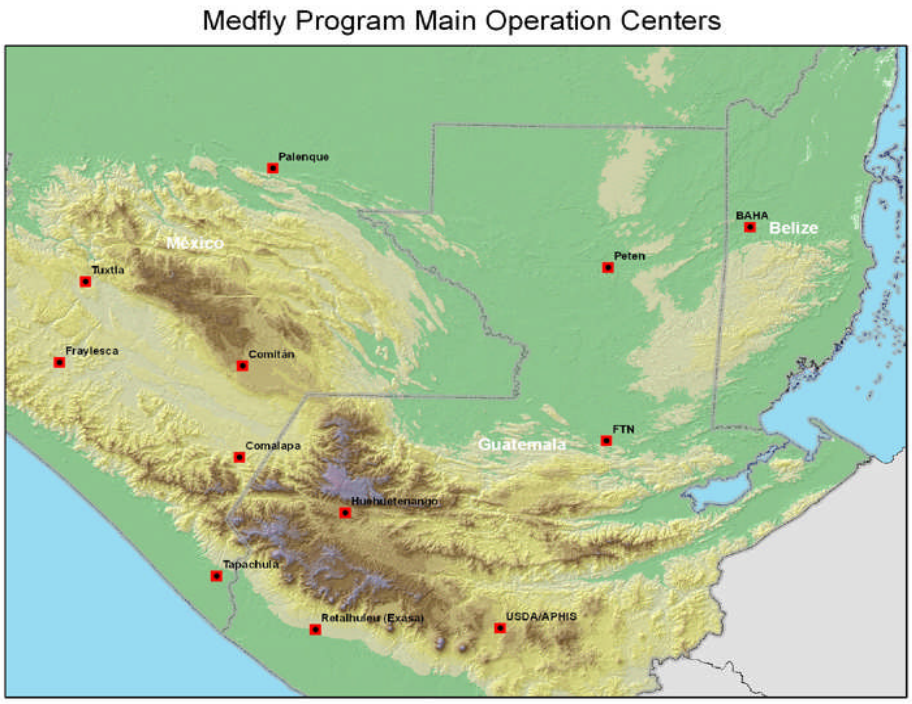

Figure 3. Medfly's operation centers in Mexico and Guatemala.

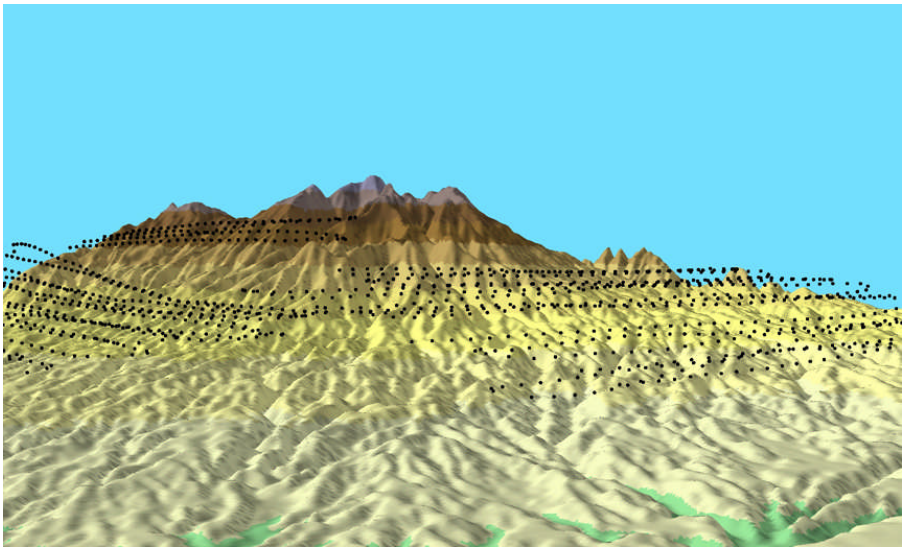

Figure 4. Three Dimension view of a flight

Resúmenes de Posters Presentados /Abstracts of Presented Posters

Monitoreo de Moscas de la Fruta del Género *Anastrepha* (Diptera: Tephritidae) en nueve Municipios del Valle del Cauca, Colombia

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Los adultos de moscas de la fruta fueron colectados en diversos cultivos presentes en nueve municipios del departamento del Valle del Cauca (Andalucía, Caicedonia, La Unión, Restrepo, Roldanillo, Sevilla, Toro y Zarzal), con el objetivo de (1) identificar las especies de *Anastrepha*, y (2) determinar las especies con poblaciones más frecuentes y numerosas. Las moscas fueron capturadas en trampas plásticas o de vidrio tipo McPhail con atrayente alimenticio (proteína hidrolizada), con revisiones semanales de enero de 2004 a diciembre de 2005. En total fueron capturadas 1 794 hembras de *Anastrepha* siendo identificadas diez especies: *Anastrepha distincta* Greene (1934), *A. grandis* Mcquart (1845), *A. pallidipennis* Greene (1934), *A. fraterculus* (Wied., 1830), *A. obliqua* (Mcquart, 1845), *A. leptozona* Hendel (1914), *A. mucronata* Stone (1942), *A. serpentina* (Wied., 1830), *A. manihoti* Lima (1934) y *A. striata* Schiner (1868). *Anastrepha fraterculus* fue la especie mas frecuente (49 % del total de las capturas), seguida por *A. striata* (39.5 %) y *A. obliqua* (7 %). Las siete especies restantes en conjunto representan apenas el 4.5% del total capturado. *Anastrepha striata* y *A. obliqua* fueron capturadas en los nueve municipios; mientras que *A. fraterculus* fue colectada en cinco municipios con el 90% de capturas en el municipio de Sevilla, sin que se colectara ningún ejemplar en Roldanillo, la Unión y Toro.

Poblaciones de Larvas de *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) en Cuatro Cultivares de Mango (*Mangifera indica* L.) bajo Alta Densidad y Poda Variable en Maracay, Estado Aragua, Venezuela

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Se evaluó el efecto de alta densidad de siembra y la poda en cuatro cultivares de mango en las poblaciones de la mosca de la fruta *A. obliqua* durante el período julio 2002 - agosto 2003, en el Campo Experimental del Centro Nacional de Investigaciones Agropecuarias (CENIAP – INIA). Los cultivares Haden, Tommy Atkins, Edwards, y Springfield, plantados a una densidad de siembra de 6x6m, fueron establecidos en un diseño completamente aleatorizado, con un arreglo factorial simple 4x6, donde los cultivares constituyen el primer factor y seis tratamientos correspondientes a testigo, testigo+pacobutrazol, poda, poda+paclobutrazol, poda+entresaque y poda+corte de ramas en cada uno de los cultivares como segundo factor, para 24 combinaciones de tratamientos. Para evaluar las poblaciones de larvas de *A. obliqua* se colectaron y disectaron tres frutos por cada tres

árboles de cada combinación de cultivar por tratamiento durante el período de cosecha. El cultivar Tommy Atkins presentó el mayor número de larvas de *A. obliqua*, diferenciándose estadísticamente de los cultivares Haden y Edwards. No se observaron diferencias estadísticas entre los tratamientos de poda en relación a poblaciones de larvas y cultivares. Estos resultados indican que la atracción de las moscas para oviponer está más relacionado con la atracción hacia los frutos de los cultivares presentes que a la práctica de manejo agronómico empleado.

Evaluación Poblacional de Adultos de la Mosca de la Fruta *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) en Mango (*Mangifera indica* L.) en Poda Variable en Maracay, Estado Aragua, Venezuela

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Se evaluó el efecto de la poda en el cultivo de mango de alta densidad en las poblaciones de adultos de mosca de la fruta *A. obliqua* colectados en trampas, durante el período julio 2002 - agosto 2003, en el Campo Experimental del Centro Nacional de Investigaciones Agropecuarias (CENIAP). Los cultivares Haden, Tommy Atkins, Edwards, y Springfield, plantados a una densidad de siembra de 6x6m, fueron establecidos en un diseño completamente aleatorizado, con dos tratamientos de libre crecimiento y 4 de poda en cada uno de los cultivares. Se utilizaron 12 trampas JD Eugo 97 cebadas con 250 ml de solución con el atrayente PedGo® para la colecta de adultos, 6 para árboles de libre crecimiento y 6 para árboles podados. Quincenalmente se realizaba la colecta de los contenidos y se recebaban las trampas. La identificación, sexado y contaje de los insectos colectados se realizó en laboratorio. Las mayores poblaciones de adultos se detectaron durante los meses de julio – agosto de 2003 (269 y 234) y en julio de 2004 (231) y no se detectaron adultos durante los meses de noviembre y diciembre de 2002. Se observaron diferencias estadísticas significativas entre las poblaciones de adultos colectados entre los árboles de libre crecimiento (1509) y los podados (171). Estos resultados señalan que existe un efecto de la poda en la disminución de la presencia de adultos de *A. obliqua* colectados en trampas en plantaciones de mango.

Fruit Fly Survey in Remnants of the Atlantic Rain Forest, Bahia, Brazil

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Bahia´s southern region in the coastal zone is surrounded by one of the few and largest remnants of the highly endangered Brazilian Atlantic rainforest, which is considered one of the richest biomes on earth. The southern region harbors the largest remnants of the Atlantic forest in Northeastern Brazil. The remaining forest cover is actually a mosaic of forest remnants imbedded in a matrix of cacao shaded plantations, commercial and family orchards. The southernmost region native lowland rainforest is now restricted to very small fragments in multistage processes of natural regeneration interspersed with commercial orchards, cattle farms and *Eucalyptus*, *Hevea* and *Pinus* plantations. We have been carrying out a comparative survey of tephritid fauna in three different secondary

forest areas in the state of Bahia, in Una (Estação Experimental CEPLAC), in Belmonte (Estação Experimental CEPLAC), and Ituberá (Reserva da Plantações Michelin da Bahia). Fruit flies were collected using plastic McPhail traps baited with hydrolyzed protein and set up in tree canopies from. A total of 3,899 *Anastrepha* adults was captured (2,294 females and 1,605 males) from August 2007 to May 2008. Thirteen species of *Anastrepha* were identified: *A. antunesi* Lima (1.20%), *A. bahiensis* Lima (5.67%), *A. dissimilis* Stone (0.32%), *A. distincta* Greene (61.24%), *A. fraterculus* (Wied.) (21.74%), A. *grandis* Macquart (0.13%), *A. leptozona* Hendel (4.92%), *A. obliqua* (Macquart) (0.88%), *A. pickeli* Lima (0.06%), *A. pseudoparallela* (Loew) (0.57%), *A. serpentina* (Wied.) (0.69%), and *A. sororcula* Zucchi (0.19%). Only one specimen of *Ceratitis capitata* was trapped. These are the preliminary results of an ongoing two-year survey.

Diversity of *Anastrepha* spp. (Diptera: Tephritidae) and Associated Parasitoids from Native and Exotic Hosts in the State of Bahia, Brazil

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Brazil harbors a high diversity of native and introduced fruit fly hosts and there are reports of *Anastrepha* larvae infesting hosts in 143 genera in 54 plant families. However, only 47% of *Anastrepha* species have their hosts known. In this study, we documented the fruit fly-host associations and infestation rates by systematically collecting native and introduced fruits in backyard and commercial orchards, and patches of native vegetation during 5 years in southern Bahia. Fruit were collected in multiple sites in the southern and southernmost regions of the state of Bahia, locally known as "Sul" and "Extremo Sul", respectively. The southern region harbors the largest remnants of the Atlantic forest in Northeastern Brazil. A total of 942.22 kg from 27 fruit species in 15 plant families were collected throughout this study. Of these, 15 plant species from six families were infested by *Anastrepha* species. A total of 11,627 fruit flies were reared from the fruit, all of which were *Anastrepha* (5,191 females and 6,436 males). No specimens of *Ceratitis capitata* were recovered. Eleven *Anastrepha* species were recorded, *A. antunesi* (0.04%), *A. distincta* (0.1%), *A. fraterculus* (53.6%), *A. leptozona* (4.5%), *A. manihoti* (0.1%), *A. montei* (0.8%), *A. obliqua* (33.0%), *A. pickeli* (2.1%), *A. serpentina* (1.0%), *A. sororcula* (3.0%), and *A. zenildae* (1.8%). *A. fraterculus* and *A. obliqua* were found infesting Anacardiaceae, Myrtaceae and Oxalidaceae fruit. *A. sororcula* in one species of Anacardiaceae, and four species of Myrtaceae; *A. distincta* in one species of Mimosaceae and one species of Myrtaceae, *A. serpentina* in two species of Sapotaceae, and the remaining species were associated with only one host. Three species of parasitoids (Hymenoptera: Braconidae) were found to parasitize larvae of nine species of *Anastrepha*. We obtained 1,240 braconids, *Doryctobracon areolatus* (n= 1,010, 81.4%), *Utetes anastrephae* (n=153, 12.3%) and *Asobara anastrephae* (n= 77, 6.2%).

Diversidad de *Anastrepha* spp. (Diptera: Tephritidae) en la Región Sur y Extremo Sur de Bahia, Brasil

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La identificación de las especies de moscas de las frutas que ocurren en una región es de fundamental importancia para acciones de manejo integrado, sin embargo, son pocos los estudios relacionados a la ecología básica de ese grupo en Bahia. El objetivo de este estudio fue caracterizar las poblaciones de moscas de las frutas en las regiones Sur (Ilhéus y Una) y Extremo Sur (Eunápolis e Itabela) del estado de Bahia, por medio de un análisis faunístico. Las poblaciones fueron muestradas por medio de trampas McPhail con proteína hidrolizada a un 5%, en pomares comerciales, en el periodo de noviembre de 2000 a mayo de 2004. Las trampas fueron instaladas en pomares que contenían diferentes hospederos de moscas de las frutas. Los índices faunísticos fueron calculados considerándose solo las hembras recolectadas e identificadas (8.281) del género *Anastrepha*, distribuidas en 18 especies: *A. amita* Zucchi, *A. amnis* Stone, *A. antunesi* Lima, *A. bahiensis* Lima, *A. consobrina* (Loew), *A. distincta* Greene, *A. fraterculus* (Wied.), *A. grandis* (Macquart), *A. leptozona* Hendel, *A. manihoti* Lima, *A. montei* Lima, *A. obliqua* (Macquart), *A. pickeli* Lima, *A. pseudoparallela* (Loew), *A. serpentina* (Wied.), *A. sororcula* Zucchi, *A. zenildae* Zucchi y *Anastrepha* sp.1. El mayor valor del índice de diversidad fue registrado para la región Sur (H' = 1,0209), confirmando el mayor número de especies capturadas en ese municipio (S = 18). *Anastrepha fraterculus* fue asociada a los índices máximos (clase súper) de dominancia, abundancia, frecuencia y constancia en la región Sur. Las especies *A. amnis*, *A. consobrina* y *A. leptozona* presentaron los menores índices: no dominante, rara, poco frecuente y accidental.

Species and Distribution of *Anastrepha* Schiner in the State of Tolima, Colombia

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Flies in the genus *Anastrepha* Schiner are one of the most economical important pests of the Colombian fruits. The knoledwedge of the species diversity from the any area is the first step in order to design researches looking for appropriate management technologies. In this paper the species of *Anastrepha*, their distribution and hosts in the State of Tolima, Colombia, are listed. Specimens were caught in studies carried out from 1988 to present. A total of 60.484 specimens, collected in 10 municipalities from 300 to 2.500 m.a.s.l. were identified. Specimens belonged to 24 species of *Anastrepha*. Twelve host fruits were found, incluiding new host records for species of *Anastrepha*. Only three fruits were hosts for more than one species, *Psidium guajava* (*A. fraterculus*, *A. striata*, *A. ornata*), *Psidium guineensis* L. and *Coffea arabica* L. (*A. fraterculus* and *A. striata*). The wide altitudinal distribution were found for *A. distincta*, *A. sororcula* and *A. striata* (300 to 2.200 m.a.s.l.). Quarantine importance species as *A. fraterculus*, *A. grandis* and *A.*

serpentina are not widespread in the state and They are found higher than 1.100, 960, and 900 meters above sea level respectively, meanwhile, *A. obliqua*, was found from 300 to 1,550 m.

Utility of Electroantennography for Development of Food-based Attractants for *Anastrepha* Fruit Flies

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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, field captures are variable with synthetic lures, and this variability does not appear to be related solely to 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. All EAG analyses were conducted with the Caribbean fruit fly, *Anastrepha suspensa* (Loew), of known age and physiological state. Antennae were presented with chemical samples in vapor form, and EAG responses were recorded and normalized relative to a standard reference chemical. To date, our group has quantified response to ammonia, carbon dioxide, putrescine, and a series of diamines homologous to putrescine. 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. EAG has provided insight into the antennal chemoreceptors involved in detection of semiochemicals, and has identified potential new fruit fly attractants. Information obtained from EAG analysis will be used to determine relationships between antennal sensitivity to fruit fly attractants and efficacy of those compounds when deployed in traps for capture of pest *Anastrepha* species.

Occurrence and Geographical Distribution of the Fruit Flies (Diptera: Tephritidae) in the Area Producing of Tropical Fruits in the Equator

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In the Republic of the Ecuador, the most important geographical accidents are: the Andean Mountains and the Equatorial line, those confer special characteristic and a variety and potentiality particular productive. Considered as a country in development; the evolution and economic growth

of its economy has been based in two fundamental activities: the production and export of agricultural products, with emphasis in tropical fruits. In function of this, is demanded a previous knowledge of the analysis faunístic of the tefritídeos, facing the quarantine barriers imposed by importer countriesof fresh fruits. This work tries to show the conditions of the tefritidis in two of the main producer-exporters provinces of tropical fruits in the country, Guayas and Santa Elena. Jackson and McPhail traps were used in the different trapping routes inside the county. In a 3 year-old lapse, 81.307 individuals of fruit flies were captured, from them 71,42% were of the gender Anastrepha, 28,58% to the gender Ceratitis. The first caught of the gender Hexachaeta spp., was reported in this area, previously was already reported in other province (Lopez, 2002). There were registered 15 species of Anastrepha, the Mediterranean Fly,Ceratitis capitata and the tephritide from the gender Hexachaeta sp.: A. fraterculus, are the species of more common (found in 25 host), followed by A. obliqua, A. striata, A. serpentine and A. chiclayae. For the first time we have the presence of A. macrura in the country, specie that had not been reported in references neither in previous works for Ecuador. The species concave A. was reported by Norrbom in 1985 at Los Ríos province. Ecuador possesses a total of 38 species of the gender Anastrepha and the presence of Ceratitis capitata as an exotic or introduced species.

Removal of Putrescine from a Food-Based Synthetic Attractant and Capture of Sterile Medflies

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Field trials were conducted in Guatemala and in Florida to compare capture of sterile *tsl* medfly males in traps baited with ammonium acetate, trimethylamine and putrescine with capture in traps baited with ammonium acetate and trimethylamine (i.e., putrescine deleted from three component food-based synthetic attractant). Tests in Guatemala evaluated medflies released under standard aerial protocols, and compared capture in Multilure traps and in open-bottom dry traps (baited with food-based synthetics) with capture in TML-baited Jackson traps. Low numbers of males were recaptured, with highest capture in Jackson traps (5.5 males per trap per day). Removal of putrescine significantly decreased capture of sterile males in Multilure traps, and slightly decreased capture in open-bottom dry traps. In Florida, capture of ground-released sterile males was evaluated in small-scale tests using Multilure traps only. Higher numbers of males were recaptured in these tests, and removal of putrescine had no effect. Relationships among captures in Jackson traps with trimedlure versus Multilure traps with ammonium acetate and trimethylamine with and without putrescine should be tested under target environmental conditions to determine if sterile medfly capture is sufficient to meet needs for accessing sterile fly distribution. However, deletion of putrescine in traps used for detection of new invasions may compromise effectiveness of those traps for detection of new invasions in pest-free areas. Delay in detection of new invasions will result in increased costs for control and eradication that would offset cost savings from deleting the putrescine from the synthetic attractant.

Towards a Database for the *Anastrepha* Species and Their Host Plants in Brazil

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Significant advances in studies on genus *Anastrepha* were done in Brazil since 1970´s, mainly due to a better understanding of the taxonomic knowledge of the species. This knowledge was pivotal to capacity building of many researchers, graduate students and technicians that allowed the establishment of some working groups which contribute to increase the knowledge on the *Anastrepha* fruit fly diversity in Brazil. Thus, new records of *Anastrepha* species and their host plants have been frequently found out and any effort to catalogue these data on a printed version publication becomes outdated rapidly. For example, the most recent compilation (Zucchi 2007) is already incomplete because new data were found out just after this publication. Therefore, in order to develop a dynamic catalogue for the *Anastrepha* species in Brazil which could be promptly updated, an online web-based database was implemented. Examples of information available on the database: (1) 101 species recorded; (2) 30 species exclusive; (3) records of *Anastrepha* in all Brazilian states; (4) occurrence of *A. obliqua* in 25 states; (5) four recent species records: *grandicula* Norrbom in Amazon state, *A. limae* Stone in Amapá state, *A. mucronota* Stone in Tocantins state and *A. lanceola* Stone in Espírito Santo state; (6) two recent family plant associations: *A. coronilli* from fruits of Dileniaceae and Memecylaceae; (7) three recent host plants: *A. fumipennis* from *Geissospermum laeve*, *A. nascimentoi* from *Cathedra bahiensis*, *A. parishi* from *Oenocarpus bacaba* and *A. elegans* from *Chrysophylum gonocarpum*. (8) bibliographical references for all species and host records. The database is available at www.lef.esalq.usp/anastrepha.

Faunistic Analysis of *Anastrepha* Species (Diptera: Tephritidae) in the State of Amazonas, Brazil.

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The knowledge of the entomofauna of a given region or ecosystem allows the characterization of important details to determine the insect population composition, its fluctuation over time, and the insect-plant interactions. It is one of the main resources used to determine and delimitate the communities. In order to compare the populations of species collected in McPhail traps baited with food lures in different locations in the state of Amazonas, we analyzed some faunistic indices such as frequency, dominance, constancy, and some species diversity indexes. Each location was considered a community with its own characteristics. Only the females were used in the faunistic analyses, once the taxonomy of the genus is based on the morphology of the aculeus tip. Collections were carried out from March through August 2008 in the municipalities of Iranduba, in varzea areas, Manaus and Rio Preto da Eva in non flooded forest areas, in the state of Amazonas, Brazil. The most frequent species were *Anastrepha obliqua* (66.7%), *A. leptozona* (10.4%), *A. striata* (9%), and *A. curitis* (4.2%). The remaining species showed frequency below 2%. The dominant and constant species were *A. obliqua* (91.67%), *A. striata* (70.83%), and *A. leptozona* (50%). The high

value of the Simpson (0.47) and Shannon (1.27) indices and the low value of the equitability index (0.44) indicate a high non-homogenoeus distribution of the species over the observed period. The Hill index showed that there are differences in the community composition in the sampled locations, probably due to the host community composition. The preliminary results of this analysis make a significant contribution to the Integrated Pest Management Programs which require a vast knowledge of the fauna and its relationship with the environment.

JH Treatment on *Anastrepha fraterculus* (Diptera: Tephritidae): Effect of Irradiation and Protein Content in the Adult Diet on JH Treated Males and Mass Deliver of JH.

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Sexual maturation in *A. fraterculus* males is a long process that can be shortened by the topical application of methoprene (a juvenile hormone analog). We analyzed here the effect of methoprene on irradiated males to discard any negative interaction between gamma radiation and the JH treatment. In addition, given that the ingestion of protein at adult stage increases males' sexual competitiveness in other tephritid species (including some *Anastrepha*), we addressed the effect of different protein sources on JH treated and untreated males. In order to find a method to deliver the methoprene in a fast, safe and simple way that could be transferred for its use in mass rearing facilities, we explored two methods: 1) include the methoprene into the adult diet and 2) submerge the pupae into a solution containing methoprene. We found that hormonal treatment accelerated maturation irrespectively of the fact that the males had been irradiated or not. This supports the use of methoprene as a pre-release treatment on sterile males. Protein fed males that were treated with methoprene reached sexual maturity earlier than males that were treated and fed only with sugar, or males that were fed with sugar and protein but did not receive methoprene treatment. However, some variation existed among protein sources in the capacity to accelerate maturation. Finally, we found that the two methods tested to mass deliver JH allowed the males to mature faster than untreated males. Males that were dip in a methoprene solution during the pupal stage showed a higher response to the treatment than males fed with a diet enriched with methoprene. Given the environmental problems associated with the disposal of adult diet containing methoprene, pupae dipping seems the more promising method to massively deliver methoprene to large amounts of flies.

Enhancing Mating Performance after Juvenile Hormone Treatment in *Anastrepha fraterculus* (Diptera: Tephritidae): a Differential Response in Males and Females Acts as a Physiological Sexing System

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Previous studies have shown that methoprene (a mimic of juvenile hormone) treatment reduced significantly the time required for sexual maturation in *Anastrepha fraterculus* males under laboratory conditions, supporting its use as a treatment for sterile males within the context of the Sterile Insect Technique (SIT). Here we evaluated the sexual behaviour and competitiveness of methoprene-treated males under field cage conditions, following the increase in sexual competitiveness of treated males with age. The effect of methoprene treatment on female readiness to mate was also analyzed. The study involved two strains of *A. fraterculus* from Argentina and Peru, which show a high degree of pre-zygotic isolation. We analyzed also whether methoprene treatment diminished reproductive isolation. Methoprene-treated males were equally competitive with untreated mature males, and this treatment allowed the males to become sexually competitive six days after emergence (3-4 days earlier than untreated males) but it did not accelerate sexual maturation in females or, at least, it did not induced a higher rate of mating in 7 days-old females. These results were observed both in the Argentina and the Peru strains. Methoprene did not reduce the level of pre-zygotic isolation between these two strains. Altogether, our results indicate that methoprene treatment produces sexually competitive males, even under field cage conditions. In the absence of a genetic sexing system, and even though sterile males and females of *A. fraterculus* are released simultaneously, the fact that females do not respond as males to the methoprene treatment acts as a physiological sexing effect. Therefore, in the presence of mainly sexually immature sterile females, released sexually-mature sterile males would have to disperse in search of wild fertile females, thereby greatly reducing matings among the released sterile insects and thus enhancing SIT efficiency.

Evaluation of Fruit Fly Gut Bacteria as Attractant for Fruit Fly (Diptera: Tephritidae) Management under North-Western Himalayan Conditions

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Bacteria are associated with insects belonging to all major orders of the Insecta. The insect gut provides a suitable habitat for bacteria; in return bacteria play a vital role in nutrition of its host. Permanent symbiotic associations have been known in about 15% of the insect species. In fruit flies, *Klebsiella oxytoca* and *Pantoea agglomerans* were recorded as the major bacterial symbiotes. Washed bacteria and fermented bacterial preparation were tested for their attractancy to fruit flies

under laboratory and field conditions. Washed bacterial preparation of *P. agglomerans* in combination with sugar attracted maximum number (11.33 adults/ 30 min.) of *Bactrocera cucurbitae* (Coquillett); however protein hydrolyzate in combination with sugar attracted maximum number of *Bactrocera tau* (Walker). All the combinations of washed bacteria proved superior to control (sugar alone) for both the species. *K. oxytoca* (48 h old culture) in combination with *gur* attracted maximum flies (9.33 adults/ 30 min.) of both the species when applied on potted plants. Under field conditions, *K. oxytoca* applied as foliar application and as bait in combination with insecticide resulted in significant reduction in fruit fly infestation over untreated control. The studies revealed that symbiotic fruit fly type bacteria can be exploited for pest surveillance and eco-friendly management. Specific efforts are however required to identify the attractive component from the bacteria in order to formulate symbiote based alternative fruit fly management strategy.

Post-teneral Diet Effects on Survival and Dispersal of the Mexican Fruit Fly, *Anastrepha ludens* in Chiapas, Mexico

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Anastrepha ludens, is an important fruit pest of Mexico. The Sterile Insect Technique (SIT) is an environment-friendly control method. Its success requires that irradiated insects released massively survive till sexual maturation and disperse in the field. After 6 days of post-emergence feeding with different diets (S = only sugar; P = 3:1 sugar:protein; M = mango slices; SP = 3 days S/3 days P; PS = 3 days P/3 days S; SM = 3 days S/3 days M; MS = 3 days M/3 days S), lab survival was evaluated. In addition, the effects of S, P and MS diets on survival and dispersal were evaluated in the field. Seven day old flies were released in the central point of a 7 ha mango (cv. Ataulfo) orchard near Tapachula, Chiapas, Mexico. After 2 days, 52 Multilure traps were hanged on the trees following a concentric design. Traps were serviced daily. Statistical analysis showed that the P-fed flies had a minor survival than the S and MS-fed flies both in lab and field experiments. The number of flies captured per circle showed differences between treatments. We trapped more S and MS individuals than P ones. We found statistical differences in life expectancy between sexes, but not between diets. S and MS flies reached longer distances than P ones, and the dispersal distance of females was larger than the one of males. The dispersal pattern did not differ between treatments and can not be solely explained by the wind direction.

Bioecología de *Ceratitis capitata* Wied. (Díptera: Tephritidae) en Uva "Itália" (*Vitis vinifera* L.) en el Noreste Brasileño

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Se estudiaron aspectos relacionados con el comportamiento biológico y ecológico de *C. capitata* sobre la variedad de uva de mesa "Itália" cultivada en la región del Valle de San Francisco, ubicado en el nordeste brasileño, teniendo en cuenta la relevancia del cultivo en la región, así como la reciente incidencia de moscamed sobre el mismo. En relación al comportamiento de oviposición de *C. capitata* en campo, se observaron medias de 1.8 y 4.5 perforaciones y huevos por fruto, respectivamente. *C. capitata* completa su desarrollo sobre esta variedad en 25 días. La viabilidad larval estimada y la viabilidad pupal alcanzaron valores de 15 y 57.1%, respectivamente, reflejando que se produce una elevada mortalidad durante el período larval. La fecundidad total y fertilidad de las hembras fueron de 384.6 y 64.7 % respectivamente. Los valores de las tasas neta de reproducción (16.8) e intrínseca de incremento (0.13) observadas fueron discretos. En cuanto al comportamiento de oviposición en frutos de esta variedad, pero en diferentes grados de maduración fisiológica, los resultados revelaron que las hembras ovipositaron en frutos de los cinco estados de maduración ofrecidos, con medias de 39,5 (60 días); 51,2 (70 días); 49,6(80 días); 72,3 (90 días) y 106,2 (100 días) perforaciones por racimo. La viabilidad pupal alcanzó valores medios en general de: 44.7; 43.6; 48.7, 66.3 y 54.7 % para los estados de maduración de 60, 70, 80, 90 y 100 días, respectivamente. El peso medio de las pupas por estado de maduración mostró valores de: 4,0 (60 días); 4,1 (70 días); 5,7 (80 días); 7,5 (90 días) y 10,1 mg (100 días). Los resultados presentados muestran que definitivamente la uva "Italia" representa un hospedero alternativo en la región para el desarrollo de *C. capitata*, a pesar de no constituir un substrato favorable para el desarrollo de sus estados inmaduros.

Atracción de *Anastrepha* spp. a Trampas Cebadas con Frutos Naturales en Campeche, México

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A nivel mundial las especies del genero *Anastrepha* spp., son consideradas como limitantes en la producción de frutos. En el Estado de Campeche, México, no se encuentran reportes actuales sobre el desarrollo de nuevos atrayentes para el control de las moscas de la fruta encaminados al concepto de sustentabilidad. Actualmente para el monitoreo de poblaciones adultas de tefrítidos se utilizan trampas "Multilure" cebadas con una mezcla de proteína hidrolizada, bórax y agua, sin embargo es indispensable la búsqueda de atrayentes más efectivos derivados de los frutos hospederos que sirvan como kairomonas para eficientizar la captura de moscas de la fruta. Lo anterior impulsó la realización del presente trabajo. Se llevó a cabo una serie de experimentos en una huerta de cítricos de 60 ha ubicada en Campeche, México, se encuentra localizada entre los 18° 37.346' N y 90° 55.495' WO con 37.2 msnm, presenta un clima cálido subhúmedo con lluvias en verano. Se colocaron 12 trampas Multilure cebadas con proteína hidrolizada y frutos de la región (chico zapote, naranja, toronja y mango var. Manila) con fecha del 15 de marzo al 30 de junio del 2006. El estudio fue dividido en tres fases dado que los frutos que se utilizaron se obtuvieron de la misma huerta en diferentes periodos de acuerdo a su disponibilidad; el tratamiento de proteína fue usado como testigo en todos los experimentos. La distribución de los tratamientos fue al azar, con cuatro repeticiones, la revisión y recebado se realizó cada siete días. El uso de 100 g de frutos con niveles de 9.5 y 10.5 grados ºBrix como cebo en trampas Multilure incrementa el numero de adultos capturados en las trampas, en comparación con el tratamiento testigo ($p<0.05$). El mango var. "Manila" fue el fruto con mayor atracción y la especie predominante capturada fue *A. obliqua*.

Female-Biased Host Attractants for Melon fly, *Bactrocera cucurbitae*

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The melon fly, *Bactrocera cucurbitae* (Coquillett), is a serious crop pest throughout the Pacific, sub-continental and southeast Asia, causing damage to a variety of solanaceous and cucurbit related crops. Attractants for female melon flies are of particular interest as they could be used to reduce pest levels by killing both the female and her potential offspring. Previous work has shown that freshly sliced cucumbers are attractive to female melon fly and the objective of this research was to identify a synthetic lure for *B. cucurbitae.* Coupled gas chromatography-electroantennogram detection (GC-EAD) analysis of fresh and aged cucumber slurry volatiles identified 32 compounds which were detected by melon fly females. Active compounds were initially screened as single components in glass McPhail traps in outdoor rotating olfactometer experiments. Synthetic blends were composed based on initial screening results and EAD responses and tested in both an outdoor rotating olfactometer and field cage using mated lab-reared melon flies. Six- and nine-component blends were shown to have female biased attraction in outdoor rotating olfactometer and field cage experiments. Initial field captures with the nine-component blend were two times greater than with solulys protein bait. Subsequently a seven-component blend was formulated into plastic matrix plugs and caught more females that solulys in McPhail type traps. Besides being more attractive, this lure may have several other advantages over protein baits; it has the potential to be used with a dry trap or bait station, to be long-lasting, and it captures comparably low numbers of non-targets. Applications of this new female-biased lure include trapping for detection (new infestations/biosecurity) and monitoring/delimitation (for treatment timing), as a control/eradication method with mass trapping or an attract-and-kill system, or as an attractant additive to existing protein insecticide bait sprays such as GF-120.

Jugo de Uva y Trampas Transparentes para Mejorar la Captura de Mosca Mexicana de la Fruta, *Anastrepha ludens* (Loew) (Diptera: Tephritidae)

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La detección oportuna de adultos de mosca mexicana de la fruta [*Anastrepha ludens* Loew (Diptera: Tephritidae)] en huertos de cítricos es un factor clave para iniciar las medidas de manejo de la plaga. En el presente estudio se evaluaron varios atrayentes y trampas diversas para contribuir a mejorar la detección de *A. ludens*. Los experimentos fueron establecidos en Hualahuises, Linares, y General Terán, N.L., México. Se compararon trampas McPhail, multilure transparente, y una trampa transparente desarrollada en el INIFAP. Además, se evaluaron los atrayentes: torula, acetato de amonio+putrescina+ propylene glycol, y jugo comercial de uva. Las trampas se instalaron en huertos de naranja Valencia y áreas de chapote amarillo [*Casimiroa greggii* S. Wats (Rutaceae)]; fueron revisadas y rotadas semanalmente. En el área experimental se liberaron semanalmente 20,000 especímenes estériles de *A. ludens*. Los resultados mostraron que en las trampas multilure transparentes con 350 ml de jugo de uva como atrayente se capturaron significativamente más

ejemplares de *A. ludens* en comparación con: trampa McPhail+torula, trampas multilure +torula, y trampas multilure+acetato de amonio+putrescina+propylene glycol. Las trampas transparentes multilure y la desarrollada en el INIFAP, con 350 ml de jugo de uva, mostraron una respuesta similar en la captura de adultos de *A. ludens*. Con estas combinaciones se obtuvieron capturas de la plaga desde el primer día en que las trampas fueron instaladas en los árboles. Las trampas transparentes con jugo de uva como atrayente podrían ser útiles para detectar a la plaga cuando se encuentra en niveles poblacionales reducidos. La alta efectividad en la captura de *A. ludens*, costo bajo y fácil manejo de la trampa transparente INIFAP, aunado a la notable capacidad de atracción del jugo de uva, representan una alternativa excelente para el monitoreo de *A. ludens* en México y América Latina.

Sesión/Session V

Cría Masiva y Control de Calidad de Moscas de la Fruta y Parasitoides/Mass Rearing and Quality Control of Fruit Flies and Parasitoids

Assessment of X Ray Irradiation for Fruit Fly Sterilization

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Introduction

The Sterile Insect Technique (SIT), which is used in many countries to suppress pest insect populations, uses radiation to sterilize mass-reared insects. Ionizing radiation from ^{60}Co and ^{137}Cs, that can ionize atoms and break molecular bonds, has been used extensively to sterilize insects, for example the fruit flies (Diptera: Tephritidae) (Bakri and Hendrichs, 2004). Recently, however, the shipment of these radiation sources has become difficult. Among other reasons, concern over the safety of the transport of radioactive isotopes through their regions has been raised by several countries. For example, between September 2007 and March 2008 a total of 13 delays and denials of shipment of ^{60}Co sources was reported by the International Irradiation Association in its May 2008 newsletter, which reported on outcomes from an International Atomic Energy Agency workshop convened to debate this issue.

As a result of these problems, a survey of alternative sources of irradiation that could be used to sterilize insects was initiated. One such alternative is X rays (Bakri *et al.*, 2005). The main advantage of this alternative is the ability to ship these sources around the globe without any special requirement, and with a significant reduction in shipping costs. Early studies conducted with X rays in the early 1950s with screwworm (Bushland, 1953) demonstrated the feasibility of using X rays to sterilize insects for SIT purposes.

As part of this approach, a prototype X ray unit was tested at the FAO/IAEA Agriculture and Biotechnology Laboratory (Seibersdorf, Austria) with the objective to evaluate its suitability as a replacement for gamma irradiation. The X ray prototype (RS2400) has its own external water cooling unit to remove the waste heat from the X ray tube. It is designed to irradiate fruit fly pupae in batches of about 20 L, as the irradiation chamber houses five cylindrical canisters, each measuring 178 mm (internal diameter) by 200 mm (length). Each irradiation canister is suspended in a cradle so that it can revolve around the horizontal X ray tube at a distance of about 10 mm from the surface of the tube, providing radiation to all sides of the canister. Initial studies on dose-mapping of the X ray unit in this configuration indicated poor radiation distribution with a resultant potential for uneven irradiation dosage.

Dose rate and dose uniformity ratio (DUR) were major issues that needed to be addressed during the refinement of the prototype. An improved canister was developed, blocking five centimetres from the edges of the canister, without a substantial decrease in the total volume available for pupal irradiation (about 18.7 L). In addition, 0.5 mm of steel were incorporated into the wall to filter the X ray beam, producing a substantial improvement in the dose distribution (DUR < 1.3), whilst maintaining an acceptable dose rate (Fig. 1).

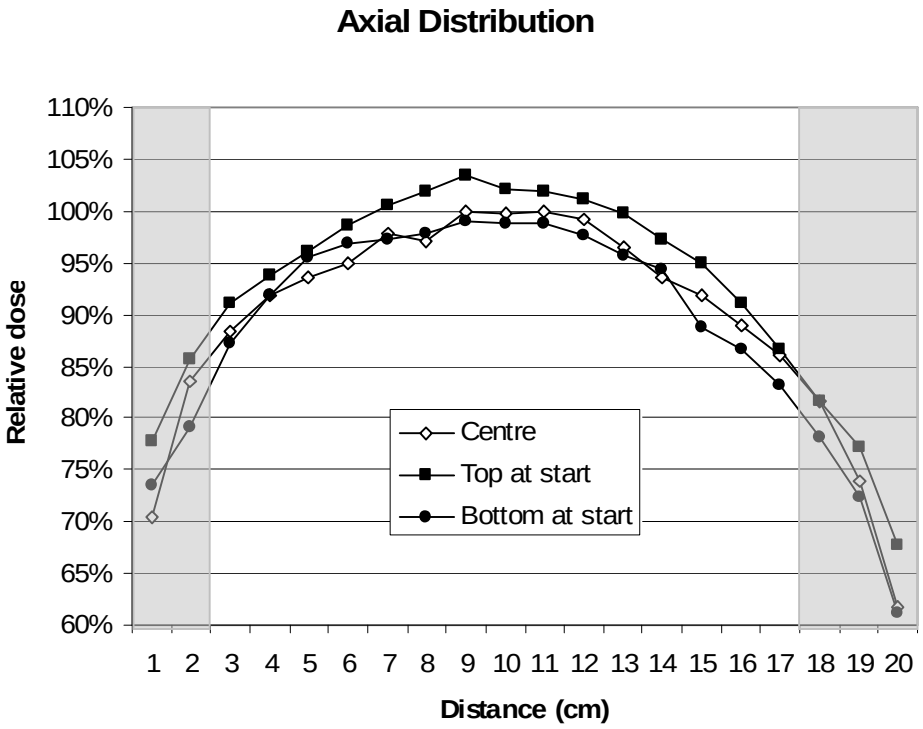

Figure 1. Axial dose distribution in the X ray irradiation canister with 0.5mm steel filtering. Radial dose variation is less than 5%. The irradiation volume is restricted to the middle 150 mm of the canister (non-shaded area) to achieve a DUR < 1.3.

Another issue with the original prototype was that the final dose rate (about 11.5 Gy min^{-1}) was lower than initially expected. However, in view of the large volume that can be irradiated at one time, the lower dose rate can be tolerated. Issues that remain to be addressed concern the reliability and life span of the X ray tubes, increasing the available dose rate, measurement and control of the dose received by the insects and calibration of a suitable dosimetry system for routine process control.

The radiation dose that is used to induce sterility in insects is of critical importance for SIT programmes. Insects that receive a dose that is too low have a high residual fertility and are unsuitable for SIT proposes; however, too high a dose will result in insects that do not compete well against wild insects in the field (Parker and Mehta, 2007). For this reason, a series of quality control tests were conducted according to the FAO/IAEA/USDA (2003) Quality Control Manual, to compare the impact of the X ray irradiation with the standard gamma irradiation procedure (^{60}Co source) on tephritid fruit flies. These tests included: sterility (i.e. residual fertility) test; percent emergence and flight ability; longevity under stress; and mating performance in field cages. Two fruit fly species were tested with different doses and controls. *Bactrocera cucurbitae* (doses: 20, 30, 40, 50 and 70 Gy); *Ceratitis capitata* (doses of: 15, 30, 60, 90, and 120 Gy). Preliminary data show that the mating performance in field cage tests and the other quality control parameters are identical when irradiated with X or gamma rays for the experiments completed so far.

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The Role of Microorganisms in Enhancing Reproductive Success of the Mediterranean Fruit Fly *Ceratitis capitata* (Diptera: Tephritidae)

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Introduction

Mediterranean fruit flies (*Ceratitis capitata*, Diptera: Tephritidae), harbor a community of diazotrophic bacteria in their digestive system. Until recently, the association between the Mediterranean fruit fly (medfly) and bacteria received little scrutiny (see Drew and Lloyd 1991, Lauzon 2003 and Behar *et al.* 2008a) for reviews of tephritid-bacteria interactions).

Marchini *et al.* (2002), using culture dependent methods, studied the bacteria associated with the oesophageal bulb of the medfly. They found that this organ harbors a discrete community of bacteria, comprised mainly, as in other fruit flies, of members of the family *Enterobacteriaceae*. Similarly, a systematic study of the structure and diversity of microbial communities in eggs, larvae, host fruit, pupae and adult medflies, based on 16S rDNA sequences obtained from PCR-DGGE and from isolated colonies revealed that members of the *Enterobacteriaceae* constitute the dominant populations in the medfly's gut. Most prominent were species of *Klebsiella,* which were found in different combinations with *Citrobacter freundii*, *Enterobacter* spp, *Pantoea* spp., *Pectobacterium* spp. and *Providencia stuartii* (Behar *et al.* 2005, 2008a,b).

These analyses also found that, as in other fruit flies, *Klebsiella oxytoca* is the most common species in the medfly's gut. Furthermore, a species of *Pectobacterium* was found to be commonly associated with the medfly, particularly in the larval stages. PCR-DGGE analyses also supplied evidence that the enterobacterial community, composed mainly of *K. oxytoca*, *P. cypripedii*, *Pantoea* spp. and *Citrobacter freundii*, is present during all of the fly's developmental stages and some elements are vertically transmitted from the female parent to its offspring during oviposition (Behar *et al.* 2008b). Strikingly, all these bacterial species are potential diazotrophs. In addition, *K. oxytoca*, *Pantoea* spp and *P. cypripedii* are also known pectinolytic bacteria. Further to these ubiquitous bacteria, we also found small but persistent populations of potentially pathogenic bacteria, *Pseudomonas* spp., in wild and lab reared flies (Behar *et al.* 2008c).

These findings prompt the obvious question – are the bacteria that form the major populations contributing to the fitness of the fly host? Below, we briefly review a number of recent studies aimed at answering this question. In the first study, we cleared bacteria from the guts of flies and monitored female oviposition and male copulatory success. In a second series of studies, we investigated how inoculating flies with bacteria affects their longevity and, in the case of sterile males, copulatory success.

1. Effect of gut Bacteria on nutritional status and reproductive success

We recently studied the contribution of gut bacteria to fly fitness (Ben-Yosef *et al*. 2008). After clearing bacteria from the guts of adults, we were able to test the hypothesis that bacteria contribute to reproductive success of medfly adults by enhancing copulatory success in males and egg production in females. To do so, the reproductive success of antibiotic treated flies - maintained on either nutrient rich or nutrient poor diets - was compared to that of flies containing a full complement of enteric bacteria. Following eclosion, flies were fed a full diet containing peptides,

sugar and minerals, or a sugar diet, lacking peptides. Sub groups from each diet were fed a mixture of the antibiotics ciprofloxacin and piperacillin. We quantified the presence of bacteria, food consumption, weight gain, lipid and protein levels, oviposition in females and copulatory success of males in the four treatment groups. The antibiotic treatment effectively cleared the gut of bacteria. Relative amounts of food consumed - with or without antibiotics - were similar in all treatment groups. The antibiotics did not inhibit feeding, and their ingestion did not affect dry weight or the amount of protein stored, yet females feeding on the full diet without antibiotics had increased lipid levels. Females fed the full diet produced significantly more eggs than females on the sugar diet, but the presence of bacteria did not affect numbers of eggs produced. However, in the absence of bacteria, oviposition rate of nutritionally stressed females was significantly accelerated. The presence of bacteria conferred a marginally significant mating advantage to both sugar fed (P = 0.09) and protein fed males (P = 0.08), when competing with antibiotic treated males that were fed on the same diet. In sugar fed males, the addition of antibiotics did not affect the latency to copulate (P = 0.907). Conversely, in the males fed a full diet the presence of bacteria was significantly associated with a shorter latency to mate (P = 0.033; see Ben-Yosef *et al.* 2008 for full details). The diet-bacteria interaction had another manifestation, as it significantly affects longevity. Treating males and females with antibiotics affected their longevity in a diet dependent fashion: prolonging life when flies were fed with sugar, yet having no effect when combined with the full diet (Ben-Yosef *et al.* 2009). These results provide several important clues about the effect of antibiotics on the flies and on the nature of the interaction between the fly and its gut microbiota. The diet dependent prolongation of longevity elicited by suppressing the gut microbiota indicates that the nature of fly-bacteria interaction was dictated by the diet. The fact that bacteria were associated with a decreased longevity only in sugar fed flies suggests that antagonistic bacterial activity was induced in response to unfavorable environmental conditions present only in the gut of these flies. Alternatively, qualitative or quantitative changes in the species composition of the gut bacterial community, induced by the diet, could have been the cause of a different net effect on the longevity of the host fly. Thus, the gut microbiota may be alternating between mutualism / commensalism and parasitism in response to the composition of their host's diet.

2. Effects of bacterial inoculation on fly longevity and copulatory success

This experiment assessed the effect of the dominant Enterobacteriaceae community and the minor *Pseudomonas* community on the medfly's longevity (Behar *et al.* 2008c). Ingestion of bacteria significantly affected mortality rate and average fly longevity in both sexes, regardless of previous exposure to antibiotics. In general, feeding on *Pseudomonas* had a negative effect on longevity, while feeding on the Enterobacteriaceae mixture had a beneficial effect. Compared to flies fed on sugar alone, flies fed on *Pseudomonas* died at a significantly faster rate and their average longevity was significantly reduced. Conversely, flies fed on Enterobacteriaceae died more gradually and lived significantly longer, on average, than flies fed on sugar. Thus we concluded that the dominant presence of the enterobacterial community in the medfly's gut contributes to its longevity. Previous work has shown that this community is capable of contributing to the fly's nitrogen and carbon metabolism, development and copulatory success. In addition, we suggest that the Enterobacteriaceae community within the medfly's gut also has an indirect contribution to its host fitness by acting (through an as yet unknown mechanism) as a barrier against deleterious bacteria (Behar *et al.* 2008c).

Finally, we attempted to improve the performance of sterile males by inoculating them with a suspension of *Klebsiella oxytoca*, one of the most commonly found bacterial species in the medfly gut. Significantly, sterile males inoculated in this manner copulated faster (i.e., exhibited a shorter latency to mate), than sugar fed sterile males or sterile males fed a suspension of autoclaved *Klebsiella oxytoca* (Ben-Ami 2008).

Conclusion

Taken together these results encourage us to continue to investigate the precise manner of the contribution of bacteria to the fitness of the medfly and other tephritids. In addition, we suggest that supplementing the post teneral diet of sterile males with beneficial bacteria can significantly improve the effectiveness of the sterile insect technique.

Acknowledgement

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Resúmenes de Posters Presentados/Abstracts of Presented Posters

Alternative of Wheat Germ Oil as a Supplement in Fruit Fly Liquid Larval Diet

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One of the most important components in the SIT (Sterile Insect Technique) program for suppression fruit fly population worldwide is to have a well-established rearing diet and methodology to mass rear fruit flies. USDA-ARS recently developed a cost effective liquid diet for rearing fruit fly larvae. The fruit fly quality from rearing larvae in this liquid diet has been satisfactory. This liquid diet technology has been transferred to more than 36 interested groups with more than 26 fruit flies or other insect species worldwide for evaluation. However, one of the hindered factors for implementing liquid diet technology has been lacking resource of wheat germ oil either too pricey or the availability in locality. Our objective is to find a cheaper and more available alternative to replace wheat germ oil. In this study, four alternative oils (corn oil, vegetable oil, canola oil with 10% vitamin E, and canola oil with 20% vitamin E) from a Hawaii local supermarket, in addition to two controls (wheat germ oil and no oil addition) were substituted for wheat germ oil and were evaluated based on pupal recovery (%), larval duration (d), pupal weight (mg), adult emergence (%), adult fliers (%), mating (%), egg production per female per day, egg hatch (%), and peak egging period (d). The results showed that there were not significant differences in adult emergence, adult flier, and peak egging period among these six treatments. A significant difference in pupal recovery, larval duration, or pupal weight between no oil and the other five oils was also observed. Percent mating from wheat germ oil, corn oil, and canola oil with 20% vitamin E took the lead while vegetable and canola oil with 10% vitamin E made the second. Mating stayed the lowest from the diet without oil. Adults reared as larvae in the liquid diet containing wheat germ oil, corn oil, or canola oil with 10% vitamin E produced significantly more eggs than those reared in the diet containing no oil, vegetable oil, or canola oil with 20% vitamin E. All eggs were equally viable except those from rearing in the diet containing no oil. Therefore, we concluded and recommended that corn oil is the best alternative to replace the wheat germ oil.

Influencia de la Densidad de Adultos, Densidad de Larvas y Tiempo de Exposición en la Cría Masiva de *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae)

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La influencia de la densidad de adultos, densidad de larvas y tiempo de exposición fueron evaluadas. Tratamientos de 2400, 3000 y 3600 adultos/jaula; 1600, 2300 y 3000 larvas/unidad de parasitación; y 1, 1.5 y 2 horas de exposición se aplicaron en cría masiva. Las evaluaciones se hicieron para cada una de las tres sesiones de exposición a parasitación (primera exposición, segunda exposición y tercera exposición) realizadas de manera consecutiva durante el día, en el

proceso de cría masiva. Los parámetros de evaluación fueron superparasitismo (número de cicatrices/pupario), porcentaje de emergencia y proporción sexual (♀:♂). Los resultados fueron analizados en cada exposición con un diseño trifactorial. En la primera exposición el superparasitismo se incrementó con el aumento del tiempo de exposición (5.61 ± 0.31, 7.78 ± 0.44 y 9.69 ± 0.47 cicatrices/pupa, respectivamente) y se favoreció la proporción sexual (2.7 ± 0.15, 3.25 ± 0.20 y 3.65 ± 0.23 ♀/♂) sin afectar la emergencia, misma que fue más baja en la menor densidad de larvas (48.9 ± 1.11, 52.78 ± 1.18 y 51.89 ± 1.21). Tanto en la segunda como en la tercera exposición, el superparasitismo fue mayor con el aumento del tiempo de exposición. El incremento en la densidad larvaria redujo el superparasitismo y la proporción sexual en la segunda exposición. No obstante en la tercera exposición la proporción de hembras fue favorecida cuando se incrementó la densidad larvaria. En ninguna exposición se presentó un efecto significativo de la densidad de adultos ni de la interacción de los tres factores. De acuerdo a los resultados se podría optimizar la cría utilizando 2400 adultos/jaula de cría, 3000 larvas/unidad de parasitación y 1 h de exposición.

Cría Masiva del Parasitoide de Pupa de Moscas de la Fruta *Coptera Haywardi* (Hymenoptera: Diapriidae)

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Coptera haywardi es un parasitoide que ha demostrado amplia especificidad hacia pupas de moscas de la fruta. Esto le confiere ventajas para su aplicación e integración dentro del Control Biológico de esta plaga. Por consiguiente se establecio su cría masiva para usarla y reforzar las liberaciones aumentativas de *D. longicaudata* (parasitoide de larva). El método de cría se lleva a cabo en jaulas agrupadas en una estructura modular. Cada modulo consta de 24 jaulas distribuidas en dos secciones (12 jaulas por cada sección), con dimensión de 40 x 30 x 100 cm por jaula. Las partes superior y costados del modulo son cubiertas por malla para evitar la fuga de las jaulas. La parte anterior y posterior de la jaula es cubierta con acrílico, con puertas abatibles para la entrada y salida del material biológico. Cada jaula dispone de un tubo bebedero y el alimento a base de miel es adicionada en las paredes laterales del modulo. La jaula tiene una capacidad para albergar aproximadamente 6,000 parasitoides (2 ♀: 1 ♂). Diariamente se introducen, en la parte anterior de cada jaula, 2,000 pupas hospederas sometidas previamente a irradiación. Los hospederos permanecen 72 horas expuestos, posteriormente ser retirados por la parte posterior del modulo. El tiempo de producción de cada modulo es de 15 días y durante este periodo permanecen en el área de colonia a 23 ± 2° C. Los hospederos parasitados son colocados en charolas de fibra de vidrio (77 x 40 x 6 cm) en proporción de 1 lt de pupa (25,000 pupas) con 1 Lt de vermiculita, permaneciendo 30 días a temperatura de 26 ± 2° C y 60 – 80 % de H.R. Con la metodología usada se ha alcanzado un porcentaje promedio de emergencia del 65 % y una producción promedio semanal de 300,000 pupas por módulo.

Establishment of a Fruit Fly Parasitoids Mass-Rearing Facility in the Province of San Juan, Argentina

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Since 1986, the suppression strategies implemented against *Ceratitis capitata* through the National Fruit Fly Control and Eradication Program in the province of San Juan (ProCEM San Juan), have been based on integrated use of SIT, cultural and chemical controls, plus a quarantine system. In April/2008, the Biological Control has been incorporated into control activities of the ProCEM San Juan. The first step was the establishment of a colony of the parasitoid *Diachasmimorpha longicaudata* on third-instar larvae of tsl *C. capitata* strain VIENNA 8 at the San Juan facility. The rearing cages of *D. longicaudata* were held in a rearing room with 24 ± 1 ºC, 65 ± 5% RH, and 12:12 (L:D) h photoperiod. This parasitoid colony was derived from a strain previously reared on late-third instar *Anastrepha fraterculus* larvae at the Insectary of PROIMI-CONICET, Biological Control Division, located in San Miguel de Tucumán, Argentina. However, the colony of *D. longicaudata* introduced to Argentina was obtained from a strain already adapted to laboratory conditions using *Anastrepha ludens* (Loew) larvae as a host in the Biological Control Laboratory of the Mexico's Moscamed-Moscafrut National Program in Metapa de Dominguez, Chiapas, México. Financial support to introduction and establishment of *D. longicaudata* in Argentina was provided by the Agencia Nacional de Promoción Científica y Tecnológica, Ministerio de Ciencia, Tecnología e Innovación Productiva de Argentina (Grants PICT/97 n° 01236 and PICTO/02 n° 12909). Currently, quality control parameters for *D. longicaudata* reared on *tsl C. capitata* strain is being analyzed in order to evaluate the rearing process and the final product. In a second phase, is planned a mass rearing of *D. longicaudata* to reach a weekly production of 5 million parasitoids. In a third phase, the parasitoids will be released in some ecologically isolated fruit-growing areas of San Juan in combination with sterile Medfly releases in order to evaluate parasitoid efficiency once released in the field. Medfly eradication might be reached in those areas with the use of environment-friendly technologies such as SIT and Biological Control.

¿Es el Tamaño del Hospedero un Indicador de la Calidad en la Cría Masiva de *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae)?

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Diachasmimorpha longicaudata (Ashmead) es un endoparasitoide de moscas de las frutas criado y usado como un agente de control de varias especies de *Anastrepha* en México. Durante la producción masiva de *D. longicaudata* diferentes tamaños de individuos son producidos dependiendo del tamaño del hospedero. En éste trabajo se investigó si el tamaño del hospedero influye sobre los parámetros biológicos y comportamentales de los parasitoides emergidos. Tres diferentes tamaños de hospederos (pequeños, medianos y grandes) fueron usados para los experimentos. El tamaño del hospedero afectó significativamente el número de parasitoides emergidos ya que un mayor número de individuos emergió de los puparios de tamaño medio en comparación con aquellos emergidos de los puparios de tamaño pequeño y grande. La proporción de hembras/machos no fue significativamente diferente entre los tres grupos. El tamaño del hospedero influyó en la supervivencia de los parasitoides hembras y machos sin alimento, ya que los parasitoides grandes vivieron más tiempo. En contraste, el tamaño del hospedero no influyó sobre la supervivencia de los parasitoides a los cuales se les proporcionó alimento *ad libitum*. El tamaño del hospedero afectó la fecundidad de las hembras emergidas, las hembras provenientes de hospederos medianos y grandes fueron mas fecundas. El tamaño del hospedero no afectó los parámetros comportamentales de los parasitoides emergidos, evaluados ante la presencia de mango infestado, excepto el tiempo de aterrizaje sobre el hospedero. Las hembras provenientes de hospederas grandes tardaron menos tiempo en aterrizar en el fruto, en comparación con las hembras provenientes de hospederas pequeñas. El tamaño del hospedero no afectó los diferentes índices de trayectoria evaluados, excepto la velocidad promedio de caminar de *D. longicaudata*, las hembras provenientes de hospederos grandes mostraron un incremento en su velocidad de caminar en comparación con las hembras provenientes de hospederos chicos y medianos.

Quality Control Parameters of *Diachasmimorpha longicaudata* Reared on *Anastrepha fraterculus* Larvae

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Quality control parameters of *Diachasmimorpha longicaudata* reared on third instar *Anastrepha fraterculus* larvae at 26 ± 1 ºC, 75 ± 5% RH, and 12:12 (L:D) h photoperiod were analyzed in order to evaluate the rearing process and the final product in an experimental rearing of this fruit fly parasitoid. The study was performed in the Insectary of PROIMI-CONICET, Biological Control Division, San Miguel de Tucumán, Argentina. The colony of *D. longicaudata* used for experiments originated from a strain adapted to laboratory conditions using *Anastrepha ludens* larvae as a host and was obtained from the Biological Control Laboratory of the Mexico's Moscamed-Moscafrut National Program, Chiapas, México. Financial support to introduction, establishment and study of *D. longicaudata* in Argentina was provided by the Agencia Nacional de Promoción Científica y Tecnológica, Ministerio de Ciencia, Tecnología e Innovación Productiva de Argentina (Grants PICT/97 n° 01236 and PICTO/02 n° 12909). Approximately 140 host larvae were exposed to 35 parasitoid females for 1 h, 1.30 h, and 2 h each three days. The parental female productivity and fecundity, offspring sex ratio, % parasitoid emergence, % parasitism, daily survival and longevity

of parental females and males, and host larval and pupal mortality were evaluated for every maternal parasitoid age interval (1-3, 4-6, 7-9, 10-12, 13-15, 16-18, 19-21, 22-24, 25-27, 28-30, 31-33 age days). Each treatment was replicated three times. There was no statistical difference between most of the parameters obtained with different host exposure times to parasitoid. Higher fecundity were found at 7-9 and 10-12 maternal age intervals (2.4 – 2.6 female offspring by parental female). Female biased offspring were always found for all host exposure times. The results of this preliminary study on quality control parameters of *D. longicaudata* will be helpful in the near future to develop an optimum mass-rearing system using *A. fraterculus* larvae as host.

Recuperación de la Fecundidad, Fertilidad y Calidad de *Anastrepha obliqua* bajo Condiciones Relajadas en el Sistema de Cría Masiva

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La planta Moscafrut ha producido millones de moscas estériles de *Anastrepha obliqua* durante los últimos 10 años, tiempo en el cual solo una vez se han introducido individuos silvestres como una forma de rejuvenecer la colonia. Existe evidencia que las moscas han sido seleccionadas para reproducirse a edades tempranas, presentar menor longevidad y cierto nivel de incompatibilidad sexual (Miyatake, 1998. Res. Popul. Ecol. 40: 301:310). Para resolver tal desventaja se ha propuesto manejar las colonias bisexuales en condiciones relajadas de baja densidad, luz de día, mayor proporción de machos que hembras, temperatura óptima, incremento de áreas de descanso utilizando insertos en las jaulas de cría (Liedo et al. 2007. Florida Entomol. 90: 33-40). Por ello el objetivo de este trabajo fue determinar los parámetros de producción y calidad de una colonia de *A. obliqua* mantenida durante nueve generaciones en condiciones relajadas. El establecimiento de las colonias fue a partir de pupa obtenida del módulo de producción masiva. Las condiciones relajadas consistieron en cargar las jaulas de cría con 60, 000 pupas en vez de 88,000 y mantenerlas a 26 ±1°C y 70±5 % HR. La densidad de siembra fue de 3.7 larvas/por g de dieta. Las charolas después de haber sido sembradas permanecieron durante 8 días a 26±1^{o}C; la larva madura fue separada disolviendo la dieta en agua. La pupación fue a 20±1^{o}C durante 24 horas, después fue mantenida a 26^{o}C y 80% HR durante 13 días. Finalmente la pupa fue seleccionada para el retorno de colonia. Los resultados indicaron que la baja densidad de confinamiento de adultos en jaulas de producción masiva incrementa la producción de huevo por hembra por día de 37 a 42 aunque no de manera significativa. Las condiciones relajadas favorecieron el incremento de la transformación de huevo a larva de 81 a 84%, peso de larva de 17 a 18.5 mg, peso de pupa de 13 a 13.8 mg, emergencia de 93 a 95% y voladoras de 88 a 90%.

Determinación del Número de Huevos por Mililitro usado en el Proceso de Cría de Dos Especies del Género *Anastrepha* (Diptera: Tephritidae)

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Anastrepha ludens (Loew) y *Anastrepha obliqua* (Macquart), conocidas como mosca mexicana de la fruta y mosca de la fruta de las indias, son plagas en México en cultivos de cítricos y mango. Por

el daño económico que infringen, se han desarrollado diversos métodos de control. En la actualidad su control se realiza a través de programas de manejo integrado de plagas (MIP), que es aplicado a nivel regional para el establecimiento de áreas libres de plagas. Los métodos de control más comunes usados han sido la aplicación de cebos tóxicos (e.g., malatión-proteína hidrolizada) y la Técnica del Insecto Estéril (TIE). Esta última, por ser ambientalmente amigable, ha sido usada con éxito por muchos programas de control de plagas. La TIE consiste en la cría, esterilización y liberación de un gran número de insectos, donde los machos deben cortejar y copular hembras silvestres para lograr así la supresión de la especie. Sin embargo, este proceso requiere de metodologías precisas que permitan cumplir con las metas propuesta. En los procesos de cría se cuenta con mediciones predeterminadas que sirven como base para extrapolar la producción a niveles masivos como huevo por mililitro, larvas por kilogramo y pupas por kilogramo. El número de huevos por mililitro, sirve de referencia para determinar cantidad de huevos obtenidos por hembra, por jaula de producción y pos día. En la planta Moscafrut, de acuerdo a las metodología de cría masiva de cada especie, se estableció la evaluación de la cantidad de huevos de *A. ludens* y *A. obliqua* presentes en un mililitro de goma guar con el propósito de que los resultados sean aplicados en los procesos. Los resultados de este estudio indicaron que para el proceso de producción de *A. ludens* el número de huevecillos por mililitro es de 24,200 y para *A. obliqua* de 18,600.

Determinación de Parámetros Fisicoquímicos y Microbiológicos del Alimento Larvario de *Anastrepha ludens* y *A. obliqua* en la Cría Masiva de la Planta Moscafrut.

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En la Planta de Cría y Esterilización de Moscas de la Fruta y Parasitoides (Moscafrut) se produce masivamente *Anastrepha ludens* y *A. obliqua*. Para obtener insectos competitivos con sus relativos silvestres se requiere que los ingredientes que conforman el alimento cumplan con los estándares de calidad establecidos y satisfaga los requerimientos de cada especie. Estos estándares además deben permitir que se mantengan condiciones ambientales y de manejo adecuadas para el personal encargado de la cría. El monitoreo y control de las características mencionadas lo realiza el Departamento de Control de Calidad a través de los laboratorios de Fisicoquímicos y Microbiológicos. En este trabajo se consideraron los datos obtenidos de los análisis al alimento larvario de 2003 a 2007. Dentro de las pruebas fisicoquímicas se incluyeron el porcentaje de humedad, potencial de hidrógeno (pH) y acidez titulable; y pruebas microbiológicas como cuenta total de bacterias, cuenta de hongos, levaduras y coliformes. Las pruebas de laboratorio se realizaron a uno y cinco días de elaboración del alimento, estos puntos como indicadores del crecimiento microbiano y del cambio de textura inducido por el desarrollo de la larva. Los datos se analizaron con el paquete estadístico Stat View Versión 5 mediante la prueba de media rango para datos fisicoquímicos y microbiológicos, estos últimos previamente se transformaron logarítmicamente. Los valores de referencia obtenidos indican una notable disminución en la humedad del alimento para ambas especies, lo cual, bajo las condiciones actuales beneficia los parámetros de producción (rendimiento larvario y transformación huevo-larva). Las evaluaciones fisicoquímicas y microbiológicas de ingredientes y alimento larvario permiten asegurar la calidad de los insectos. Por lo que es indispensable la supervisón en esta etapa de la cría masiva, con la finalidad de contar con alternativas de uso y determinar condiciones propicias para la producción de insectos.

Comportamiento Sexual de Machos de *Anastrepha ludens* (Diptera: Tephritidae) Criados bajo el Sistema de Jaulas Tipo Inserto

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Diversos trabajos han mostrado que las condiciones de cría masiva afectan el desempeño sexual de los machos estériles de la mosca Mexicana de la fruta *Anastrepha ludens* (Loew). Los efectos de selección que se origina en la colonización, provocan en los machos ciertas desventajas que las hembras silvestres pueden detectar cuando se encuentran en competencia con machos silvestres. En la Planta Moscafrut se ha implementado jaulas tipo inserto, de acuerdo con Liedo et. al (2007) para *Ceratitis capitata* (Wiedemann), quienes obtuvieron resultados favorables en el comportamiento sexual del macho. El presente trabajo tuvo la finalidad de comparar la calidad de *A. ludens* producida bajo el sistema de jaulas Metapa (M) versus jaulas Metapa modificadas (MI) (con inserto, simulando las superficies de las hojas de los árboles). Para lo cual se realizaron pruebas de competencia sexual comparando machos criados bajo el sistema de jaulas Metapa, jaulas Metapa modificadas (durante 32 generaciones) y silvestres bajo condiciones de jaulas de campo. Los resultados indicaron que el horario del llamado sexual del macho del sistema de jaulas Metapa (M) inicia y termina antes que el de los machos con sistema de jaulas Metapa modificadas(MI), sin que exista diferencia respecto al número de llamados, siendo ligeramente mayor en adultos provenientes de jaulas con insertos (MI). Se observó un mayor número de apareamientos en los insectos provenientes de las jaulas con insertos (MI), existiendo diferencia significativa entre los adultos de jaulas sin insertos (M) y con un comportamiento igual al insecto silvestre. Lo anterior permite concluir que el macho de jaulas tipo (MI) tiene mayor posibilidad de éxito de cópula con hembras silvestres que los macho criados bajo el sistema tradicional. Estos resultados sugieren que las modificaciones a las jaulas de cría pueden disminuir los efectos negativos causados por la colonización y cría masiva.

Crianza Artificial de *Anastrepha fraterculus* (Wiedemann 1830) (Diptera: Tephritidae). Viabilidad de Huevos y Modelo de Jaula

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La mosca sudamericana de la fruta, *Anastrepha fraterculus* (Wiedeman 1830), constituye una de las plagas de mayor importancia económica para nuestro país ya que causa cuantiosos daños, tanto directos, por la pérdida de producción, como indirectos, derivados de la restricción o imposibilidad de exportar productos frutihortícolas Un método potencial, no contaminante, para su control es la técnica del insecto estéril (TIE) que ha demostrado ser eficaz en nuestro país para el control de otra especie de tefrítido, la mosca del Mediterráneo *Ceratitis capitata* (Wiedeman 1829). El presente trabajo tiene por objeto contribuir a la puesta a punto de la TIE sobre *A. fraterculus*. Para ello se evaluó el porcentaje de viabilidad y volumen de huevos ovipositados por un periodo de 21 días para determinar el periodo óptimo de colecta así mismo se comparó tres modelos diferentes de jaulas para la implementación de la crianza masiva e implementación de la TIE. Los resultados indican que el porcentaje de viabilidad tiene relación directa con el volumen en un periodo de 21 días obteniéndose un valor de R2 = 0.83 por lo que se optó por analizar el porcentaje de viabilidad, según la figura 16 el periodo de colecta que favorece a la colonia es de diez días donde se tiene un

67.4 % de viabilidad y 22.4 ml de huevos. Al evaluarse el volumen de huevos y el número de huevos por hembra por día en los tres modelos de jaulas, medianas, missions y grandes, las medianas y las grandes no mostraron diferencias estadísticas sin embargo las medianas tuvieron valores mas altos con un promedio de 11.4 huevos por hembra, importante para una crianza masiva, que favorecería a la implementación de la TIE, en cuanto a las jaulas missions si mostraron diferencias significativas con los otros dos modelos y valores mas bajos.

Morphometric Differentiation between a Wild and a Mass Reared Population of *Anastrepha fraterculus* (Diptera: Tephritidae)

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The South American fruit fly, *Anastrepha fraterculus*, constitutes an important pest in Argentina and other countries within the region (as Peru and Brazil). Following the success to suppress medfly populations in Argentina by means of the Sterile Insect Technique (SIT), it has been proposed its implementation again *A. fraterculus.* SIT requires sterile mass-reared males to mate with wild females,, and therefore is essential to verify that artificial rearing do not produce changes on released males that affect their mating competitiveness. In this study, morphometric characters were considered, with the aim of detecting possible divergences between a wild population and another population originated from it, but has been reared for many years under laboratory conditions (at Estación Experimental Agroindustrial Obispo Colombres, Tucumán Province). We measured 8 morphometric traits: Thorax Length (as an indicator of global size); Head Width, Face Windth -gap between eyes, Eye Length (as indicators of head shape) and Wing Length and Width, 3rd leg Tibia and Femur Length (as indicators of fly ability). A total 260 individuals were measured and the results were analyzed by MANOVA and logistic multiple regression. Laboratory individuals were larger than wild ones, which could be related with a larval diet supplied *ad libitum*. Besides, laboratory males compared with wild ones showed larger Head Width and Eye Length and narrower wings. Laboratory females also exhibited narrower wings, but no other significant differences with wild females. Artificial selection could explain these results: flight ability should not be an important trait under laboratory conditions (where food, water, mates and oviposition substrates are very close) and, at the same time, short-distance interactions become more frequent, thus favoring a facial change as a size increase.

Quality Control in an Experimental Rearing of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) on *Ceratitis capitata* (Diptera: Tephritidae)

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As the success of a biological pest control program depends on the quality of the parasitoids produced, quality control is routinely performed in mass-rearing facilities; not so much in studies of insects under experimental conditions. However this kind of studies are very important to establish values of reference for this particular rearing, adjustments in rearing procedures, and substantial improvement in the quality of the experimental material. Quality control implies the evaluation of both, the process of production, and the product obtained - in our case, it means an evaluation of the attributes of the adult parasitoids. As part of the experimental rearing of the parasitoid *Diachasmimorpha longicaudata*, we incorporated a routine of quality control of the process (registering the weights of host *Ceratitis capitata* larvae), and of the product (registering survival - with and without food - and fecundity of the adults produced). The results obtained with insects reared at 25º C and 75% RH were compared with the values reported by Cancino *et al.* (2004) in the Quality Control Manual of this specie. Most parameters registered in our laboratory were similar to those obtained by these authors except that survival of adult parasitoids without food, which was shorter than the reference value. This difference could be explained by the difference in size of the host used to rear this parasitoid, being *C. capitata* larva significantly smaller than *Anastrepha ludens* (Loew), the species used by others. Our procedure allowed to define internal standard of quality, assuring the supply of parasitoids of adequate biological performance for other experiments in the laboratory.

Sesión/Session VI

Uso y Desarrollo de Cepas de Sexado Genético/Use and Development of Genetic Sexing Strains

Characterization of Genetically Modified *Ceratitis capitata* Wied. (Diptera: Tephritidae) to Enhance Sterile Insect Technique Programs

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Resume

Genetic engineering of insect pest species, such as tephritid fruit flies, is now a routine laboratory procedure. However, field application of genetically-modified fruit fly strains to enhance operational programs has not been accomplished. Field application requires full characterization of new strains for gene function, productivity, and field effectiveness. We describe parameters and acceptable minimum values for new strain evaluation at several levels: laboratory, operational large scale rearing, and confined field releases.

Introduction

E. F. Knipling developed a theoretical model of the sterile insect technique (SIT) in the early 1940s (Klassen 2003). SIT has since been confirmed in field programs as an effective pest suppression and control methodology for the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Enkerlin *et al.* 2003). Among successful applications are the Guatemala-Mexico regional program in operation since 1977 (Hendrichs *et al.* 1983) and the Preventive Release Program in the Los Angeles Basin, California, USA (Dowell *et al.* 2000). Current Mediterranean fruit fly SIT programs employ the Vienna-8 Mix or Vienna-7/Toliman 99 temperature sensitive lethal (*tsl*) strains (Robinson *et al.* 1999). These strains include a genetic sexing mechanism based on a recessive, temperature sensitive lethal mutation. Females of this strain carry the temperature sensitive allele in the homozygous condition and are killed when exposed to an elevated temperature of 34°C for a period of 24 hours. Males are not sensitive to the high temperature treatment because they are heterozygous, and carry the wild-type allele that is attached to the Y-chromosome via a chromosomal translocation. This *tsl* strain allows for production of a 99.9% male population for release in control programs. Inherent in a translocation-based strain is as much as a 50% reduction in viability due to chromosomal abnormalities (Caceres 2002).

Prior to release in SIT control programs, the flies must be made reproductively sterile. This involves the exposure of pupae to an irradiation source that results in atrophy of the gonads. Action programs employ cobalt ^{60} and/or cesium ^{137}, as easy and practical methods for delivering the sterilizing radiation dose. The decaying nature of radioactive sources requires costly periodic replenishment to keep an appropriate level of radioactivity. In addition, requirements that address security concerns for radioactive materials are expensive.

Pupae of the Mediterranean fruit fly *tsl* strain are marked with a fluorescent powder so that the sterile males are distinguishable from wild flies in the control area. The ability to discriminate sterile flies is critical because the presence of wild flies in a 'fly-free' area has regulatory significance. A false positive, that is misidentification of sterile flies as wild, may result in the initiation of unnecessary operational activities, e.g. increased levels of detection

trapping, increased rates of sterile insect releases, and the imposition of quarantine status. These actions are very costly from the operational standpoint as well as for the agricultural industries under quarantine.

In practice, the fluorescent dye coats the puparium so that as the adults exit this structure they come into contact with the dye from surrounding pupae. The critical feature is the head because it inflates to break open the puparium. This inflated area shrinks as the adult matures, enclosing dye particles in the head capsule. Flies captured in surveillance traps are examined individually for the presence of dye particles by means of a microscope equipped with a fluorescent light. If no dye particles are present on the body of the fly, then the head is squashed to look for dye in the head capsule. If dye particles are absent in the head capsule, confirmation of the specimen as sterile requires dissection to verify irradiation-induced atrophy of the sexual organs. Although marking with fluorescent dyes is inexpensive, the dye particles are frequently lost leading to the very labor-intensive dissection process. If the preservation quality of the specimen is poor, then misidentification of sterile flies as wild may occur.

Expected benefits

There are three key areas in SIT programs where the use of genetically-modified strains may be of considerable importance (Robinson *et al.* 2004). Transgenic approaches to the production of male-only progeny could increase productivity relative to that of current sexing systems based on a T(Y-A) translocation. Incorporation of an internal genetic marker via gene transfer technology could either eliminate marking with fluorescent dyes or act as a secondary confirmation system, thus improving the efficiency of sterile fly identification. Development of novel control technologies for introduction of lethal genes into natural populations has also been proposed as a method to reduce operational costs.

Characterization steps

Characterization of transgenic strains is based on new strain evaluation conducted on classical genetic strains and current standardized quality control measurements. The function and stability of the genetic construct and the overall fitness of the strain are measured in small-scale (1,000 per replicate) laboratory trials and medium-scale ($\geq$ five million per week) rearing evaluations. These are followed by assessment at large-scale ($\geq$ 25 million per week) mass-rearing over a minimum of six generations. It is expected that individual strains, even those with the same genetic construct, will perform differently in mass-rearing. The critical parameters in determining the production efficiency of a strain are the longevity of the female in the colony, fecundity, larva to pupa survival rate, and the eclosion rate.

The stability of the genetic construct, e.g., the sexing system, can only be determined after relatively long periods of mass-production. It is expected that determining the mutation rate would require continuous mass-rearing over a period of one to two years and quality control protocols designed specifically for the new strain. The frequency of mutation events is typically very low, however under operational production levels of several million flies per week a mutation in the genetic construct (e.g., sexing mechanism) could result in strain breakdown. Maintenance of strain integrity may require the development of specific rearing systems similar to the current Mediterranean fruit fly *tsl* Filter Rearing System (FRS) (Fisher and Caceres, 2000).

Performance under field conditions is assessed in confined releases in field cages and subsequent open field trials. Confined releases are conducted under permit from the appropriate regulatory agency in accordance with recommended bio-security protocols (NAPPO 2007). The critical

parameters for field effectiveness are longevity of the male post-release and mating competitiveness. These parameters are assessed relative to males of designated control strains, e.g., Mediterranean fruit fly *tsl* strain and wild-type strains.

Conclusions

Implementation of a new strain in operational programs is a serious, and often costly, endeavor. The decision to do so should consider enhancements in operational efficiency and bio-security as well as reductions in the cost of mass-rearing and infrastructure. Implementation of genetically-engineered strains presents a unique challenge because this is a relatively new technology. It is essential that evaluations at all levels be conducted so as to provide scientifically-sound data for the risk assessment process (NAPPO 2007).

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Table 1. Quality control and field performance parameters and minimum values for Mediterranean fruit fly transgenic strains.

PARAMETER	MEDFLY *tsl*		Transgenic	Reference
	CURRENT	BASIS (weeks)	MINIMUM	
Quality control parameters				
Mean acceptable pupal weight (mg)	7.84 ± 0.25	EP (365)	> 7.5	[b]
Mean % emergence, post-irradiation	88 ± 3	EP (365)	> 75	[b]
Mean % flight ability, post-irradiation	81 ± 4	EP (365)	> 65	[b]
Longevity under stress (% alive at 48 hr)	70 ± 12	EP (275)	> 50	[b]
Sex ratio (% male)				[b]
• Control	50			[a]
• Permissive conditions	65	EP (365)	60	[a]
• Restrictive conditions (tsl heat-treated; transgenic without tetracycline)	99.8 ± 0.4	EP (365)	95	[a]
Rate of strain breakdown (based on release stream data)	< 2%	EP (365)	< 1%	[a]
Field performance				
Mating performance post-irradiation (or equivalent)				[b]
• Laboratory mating (% males mating)	80 ± 6	EP (365)	80	[b]
• Field cages (sterility index)	0.3 - 0.4		> 0.3	[b,c]
Longevity in the field, post-irradiation LT50 (days)	4		> 4	[a]
Dispersal in the field, post-irradiation (m)	100		> 100	[d]
Egg hatch of wild female X tsl male, post-irradiation	0.01%			[a]
Egg hatch of wild female X transgenic male (with and without irradiation)			0.01%	[a]
Fluorescence marker persistente	>3 weeks		> 2 weeks	[e]
Fluorescence marker scoring efficiency	110-150 flies/hr/person		90 - 110 flies/hr/person	[e]
Fluorescence marker scoring accuracy	91% primary 9% secondary < 1% tertiary		91% primary 9% secondary < 1% tertiary	[e]

[a] EP = El Pino Medfly Production Facility`s QC records, El Cerinal, Barberena, Santa Rosa, Gautemala (unpublished); [b] Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies, Version 5.0. 2003, FAO/IAEA; [c] Garcia de Leon, 1998; [d] Rendon *et al*. 2004; [e] Guatemala MOSCAMED Program Field Operations Manual

Table 2. Production evaluation parameters and minimum values for Mediterranean fruit fly transgenic strains.

Strains	**MEDFLY *tsl***		**Transgenic**	**Reference**
Parameters	**CURRENT**	**BASIS (weeks)**	**MINIMUM**	
Egg to pupae recovery (male only)	25%	EP [a] (52)	> 40%	[a]
Pupae/kg larval diet	0.18 ± 0.01	EP (52)	> 0.18 ± 0.01	[a]
Pre-oviposition period (days)	4	EP (365)	< 4	[a]
Fecundity				[a]
# eggs / female (release colony)	14.9 ± 2.18	EP (364)	> 15	[a]
% egg hatch (release colony; permissive conditions)	67 ± 7	EP (365)	> 75	[a]
Oviposition profile (days)	10 - 14	EP (365)	10 – 14	[a]
Development period				
Colony larval development time at 25°C (days)	10	EP (365)	< 7	[a]
Male larval development time at 25°C (days)	7		< 7	[a]
% pupation at 24hr	90		90	[a]
Pupal development time at 19°C (days)	13	EP (365)	13	[a]

[a] EP = El Pino Medfly Production Facility`s Quality Control records

Development of Transgenic Strains in the Genus *Anastrepha* to Improve the Sterile Insect Technique

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The ability to genetically transform the germ-line of tephritid fruit flies is now a routine process, and presently three transposon vector systems have proven successful including *Hermes*, *Minos* and *piggyBac* (Handler, 2001). Among these, *piggyBac* is the only vector system that has been used for transgenic strain development in the genus Anastrepha, the species *A. suspensa* and *A. ludens* in particular, with preliminary data from *A. fraterculus* (Handler and Harrell, 2001; Condon et al., 2007). Initial studies focused on introducing fluorescent protein marker genes that could be used for transformant selection, as well as for effective field-detection of transformants used in release programs. Further applications of transgenic technology are now being tested that should improve existing sterile insect technique (SIT) programs including strains for genetic marking, genetic sexing, male sterilization, and new strategies for biocontrol based on conditional lethality. The efficient creation and ecologically safe use of transgenic strains intended for field release will depend on new vector systems that allow targeting of transgenes to specific genomic insertion sites, and stabilization of the transgene to prevent unintended movement within the host genome or to other organisms. With the development of these tools for transgenic strain development in tephritid species, there is considerable optimism that this technology can be used to enhance our understanding of tephritid development and behavior, and our ability to control pest populations.

Fluorescent protein marking

The creation and selection of transgenic strains have depended largely on the use of fluorescent protein (FP) marker genes that are universally expressed and detected. These same FPs, including EGFP and DsRed, can be used as markers to identify transgenic insects released into the field, replacing fluorescent powders that can be unreliable and may pose health risks. Many transgenic insects are marked by FPs regulated by the artificial 3xP3 promoter (Horn and Wimmer, 2001) resulting in eye-specific fluorescence in adults, but this promoter has not proven effective in tephritid flies including *Anastrepha* species. The polyubiquitin promoter (PUb) is more reliable thus far, allows expression in all tissues for improved detection, and fluorescence from PUb-EGFP and PUb-DsRed is highly stable (Handler and Harrell, 2001). We find that dead transgenic adults from *A. suspensa* and *A. ludens* can be reliably identified in dry or wet traps after two to three weeks.

Fortuitous integration of *piggyBac*/PUb-DsRed.T3 vectors into the Y-chromosome of caribfly and mexfly has also allowed male-specific selection. This has aided in male selection for developmental studies, and also presents the possibility for genetic sexing using commercial fluorescent embryo/larval sorters.

Stabilization vectors

To improve transgenic strains for biological control new vector systems have been developed that allow enhanced stability to maintain strain integrity and prevent transgene movement to

unintended host organisms. To stabilize transposon vectors subsequent to genomic integration, a method to delete a terminal vector sequence was developed by introducing an internal tandem duplication of the other terminal sequence. After testing the system in Drosophila (Handler et al., 2004), the pBac{L1-PUbEGFP-L2-PUbDsRed1-R1} stabilization vector was created for *A. suspensa* and *A. ludens*. This vector has a duplicated 5' terminal *piggyBac* sequence (L2) placed internal to the flanking 5' (L1) and 3' (R1) termini, with independent markers placed in between each set of termini. After transformation in both species, the L2-PUbDsRed1-R1 sub-vector was remobilized by injection *piggyBac* transposase helper plasmid (phspBac) resulting in stabilized genomic integrations of the L1-PUbEGFP sequence in the absence of the 3' (R1) terminus.

Isolation and analysis of *b2-tubulin* genes for testis-specific gene expression and sperm marking

Testis or spermatocyte-specific gene expression has been recognized as a means to improve SIT by facilitating genetic sexing, male sterility, and sperm marking in transgenic strains. A visible fluorescent marker expressed in spermatocytes can be used for larval male selection, identification of mated females in the field, and for sperm precedence studies. Lethal gene expression directed by a testis-specific promoter could confer male sterility that would provide a major advance over radiation-induced sterility. A primary candidate for testis-specific promoter regulation comes from the *b2-tubulin* gene, whose gene product is first observed in early spermatocytes where it is specifically used in the axoneme for motile sperm development. We therefore sought to isolate the *b2-tubulin* gene and its regulatory sequences from *A. suspensa* and *A. ludens*. Gene isolation was first attempted by degenerate PCR on an *A. suspensa* adult male testes cDNA library, which fortuitously isolated the 2.85 kb *b1-tubulin* gene that has a 1.5 kb intron and encodes a 447 amino acid polypeptide. Subsequent PCR using 5' and 3'RACE isolated the 1.4 kb *Asb2-tubulin* gene that has a 60 bp intron and encodes a 446 amino acid polypeptide. Using primers to conserved sequences, the highly similar *A. ludens Alb2-tubulin* gene was isolated by direct and inverse PCR. The genes from both *A. suspensa* and *A. ludens* encode identical amino acid sequences and have putative *bUEI/ b2UE2* activator sequences that reside in the 5'UTR.

To functionally identify the *Asb2-tubulin* gene, qRT-PCR showed that *Asb2-tubulin* transcript was most abundant in pupal and adult males, and specific to the testes. This was further tested in *piggyBac* transformants in *A. suspensa* and *A. ludens* having a DsRed.T3 reporter gene regulated by the *Asb2-tubulin* 1.3 kb promoter region (b2tub-DsRed.T3). Fluorescent protein was specifically expressed in testes from third instar larvae to adults, and could be detected in sperm bundles present in the spermathecae from non-transgenic females mated to transgenic males. A PCR test could also detect the transgene within sperm in individual females, demonstrating the ability to identify females that had mated to released transgenic males.

A dominant temperature-sensitive proteasome subunit mutation for conditional lethality

Conditional lethal release is a variation of the sterile insect technique (SIT) for the biological control of pest insects resulting from the release of transgenic insects carrying dominant conditional lethal genes. A potential heat-sensitive conditional lethal system uses a mutant proteasome subunit, *Prosβ2^1*, first described in *Drosophila melanogaster* as the DTS-7 mutation. Proteasomes play a critical role in eukaryotic development whereby ubiquinated proteins undergo proteolysis in a multi-subunit complex known as the 26S proteasome, which is comprised of a 20S core and 19S regulatory complexes. Missense mutations in the 20S subunits lead to the production of dominant temperature-sensitive (DTS) "poison subunits" or antimorphs that disrupt proteasome function resulting in late larval or pupal lethality at 29°C. Preliminary studies showed

that the *D. melanogaster Pros26*^{1} (DTS-5) mutation results in >90% late larval or pupal mortality in transformed medflies. To further test temperature-dependent lethality we isolated and mutated the native *AsProsβ2* gene cognate from *A. suspensa*. The *AsProsβ2* transcript isolated from pupal cDNA is 1,022 nucleotides encoding 280 amino acids with more than 70% homology to the *D. melanogaster* protein and the predicted proteins from mosquito species. To test the use of aberrant *AsProsβ2*^{1} for conditional lethal release, the *AsProsβ2* gene was mutated *in vitro* at position +723 resulting in the amino acid substitution Gly170Arg, that was then transformed into wild type caribflies. Transgenic lines homozygous for the mutant gene developed into normal pupae at similar frequencies at both 25°C and 29°C, though at somewhat lower frequencies compared to non-transgenic pupae. In tests for adult eclosion at 29°C, 70% of wild type pupae reached adulthood, while four independent transgenic lines had adult survival rates of 4% or lower, with complete lethality in one line. We have therefore demonstrated that a point mutation introduced into the *AsProsβ2* gene from *A. suspensa* is analogous to the *Prosβ2*^{1} mutation in *D. melanogaster*, resulting in a dominant temperature-sensitive lethal effect during pupal development. This temperature-dependent lethal system can potentially be used in an environmentally benign insect management program, particularly in tropical and sub-tropical regions.

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Resúmenes de Posters Presentados/Abstracts of Presented Posters

Nuevas Líneas de Sexado Genético en la Mosca Mexicana de la Fruta *Anastrepha ludens* (Diptera: Tephritidae)

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La mosca Mexicana de la fruta es una plaga de interés nacional por las cuantiosas pérdidas que causa a la fruticultura nacional. La Técnica del Insecto Estéril (TIE) ha demostrado ser altamente efectiva en el control de esta plaga; sin embargo, la liberación de hembras estériles reduce su eficiencia por lo que recientemente se inició en México la construcción de Cepas Sexadas Genéticamente (CSG) de *Anastrepha ludens* con las que es posible seleccionar solamente insectos machos mediante la inducción de translocaciones Y-A y usando como marcador genético una mutación de color de "pupa negra" (*bp*) recientemente encontrada. Para lograr este objetivo, se aplicó un protocolo que consistió en la irradiación de 150 ml de pupa silvestre a una dosis de 30 Gy en un irradiador Gammacell Co^{60} y la formación de familias cruzando un macho irradiado con hembras *bp*. Se realizaron un total de trece ensayos generando diez líneas que producen machos tipo silvestre y hembras *bp* lo que permite la separación de sexos desde los dos días de edad de la pupa. Los estudios de laboratorio de la biología comparativa mostraron que las cepas 3, 7 y 10 fueron las mas productivas, mientras que en los análisis citogenéticos se encontró que la translocacion ocurrió entre el cromosoma Y y el cromosoma 2 portador del alelo mutante *bp;* este tipo de análisis se realizó en las cepas 1 y 4, siendo esta última la que presenta un mayor daño cromosómico lo que se ve reflejado en su baja fertilidad. Con la futura incorporación de mutaciones letales a estas líneas será posible eliminar a las hembras en estadios más tempranos de desarrollo lo que permitirá obtener sistemas listos para integrarse a los programas de control.

Transformation of the Mexfly *Anastrepha ludens* (Post-integration Stabilization and Sperm Marking)

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In order to improve the sterile insect technique (SIT) several germ-line transformation systems have been developed in pest insects, tephritid fruit flies in particular. Here we describe the germ-line transformation of Mexican fruit fly, *Anastrepha ludens*, using two *piggyBac* vector constructs. The first, *pB(2x5'/PUbEGFP/PUbDsRed1)*, provides green and red fluorescence marking to the insect and allows the post-insertion excision of one end of the transposon carrying the DsRed marker, after re-injection of the helper plasmid, *phspBac*. By eliminating the single 3' vector terminus, the genomic stability of remaining 5' transposon terminus and the EGFP marker

is ensured. The second vector, *pXL-PUbEGFP/Asβ2t-DsRed.T3,* provides green body fluorescence and also allows sperm-specific red fluorescent marking, driven by a spermatocyte-specific *β2-tubulin* promoter. With the first vector construct we obtained three stabilized lines (green-only fluorescence), and have observed that EGFP fluorescence is detectable at least two months after death (completely dried). With the second vector construct we obtained five lines with good red fluorescence expression in the male testes, with fluorescent sperm observed from the testes or the spermathecae of non-transgenic females mated to transgenic males. Both transgenic strains have strong potential for use in monitoring released flies in SIT, and fluorescent sperm-marking could be used for determining the mating success of sterile males in the field.

Mutante de Pupa para el Sexado Genético de la Mosca Mexicana de la Fruta *Anastrepha ludens*: Herencia y Ligamiento

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Una mutante de coloración de pupa negra (*bp*) fue encontrada en la cría masiva de *Anastrepha ludens*, Planta Moscafrut y se exploró su potencial para integrarse como marcador genético en la construcción de líneas sexadas genéticamente de esta especie. Fenotípicamente la mutación *bp* se caracteriza por la presencia de un pupario negro; sin embargo, este gen también se expresa en otras estructuras de los insectos como los espiráculos de las larvas y el cuerpo de los adultos, *bp* es recesivo y autosómico, presenta penetrancia y expresividad alta, fertilidad y sobrevivencia larvaria ligeramente reducidas. También se analizaron las relaciones de ligamiento con diferentes mutantes anteriormente aislados y estudiados que incluyen variantes de coloración de ojos: "blancos" (*we*), "rojos" (*Re*), "violetas" (*Ve*), "amarillos" (*ye*) y del cuerpo del adulto: "ámbar" (*ab*). Los genes *we, ab, ye* son recesivos, *Re* es dominante y *Ve* es dominante y letal como homocigoto. Se hicieron cruzas entre *bp* y cada uno de los mutantes, obteniendo el híbrido de la F_1 en repulsión y enseguida la F_2, también se realizaron las cruzas de prueba directa y recíproca correspondientes. Los resultados obtenidos sugieren segregación independiente entre *bp* y *we, Re, Ve*, *ye*, *ab*. Lo cual demostró que *bp* integra un grupo de ligamiento diferente a la de estos genes. Agregando a este estudio el análisis en conjunto de las relaciones de ligamiento, se han descrito cuatro grupos de ligamiento los cuales proponemos como: Grupo A (*bp*), Grupo B (*we*). Grupo C (*Re*), Grupo D (*ye, Ve, ab*). La designación de los grupos fue de forma arbitraria y aún no se han relacionado con datos citogenéticos, sin embargo el contar con estos marcadores particulares es una valiosa información para la construcción de nuevos rearreglos cromosómicos e inducción de mutaciones letales que permitan el desarrollo de líneas sexadas genéticamente.

Sexual Competitiveness of a Transgenic Sexing Strain of the Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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The Sterile Insect Technique (SIT) for the medfly generally relies on the release of sterile flies of only the male sex. Male selection is achieved through the use of a genetic sexing strain (GSS). Transgenic sexing strains (TSS) have been developed that perform the same function of female-lethality, this time by withholding tetracycline (or related compounds) from the larval diet. The use of TSS may allow for certain problems associated with conventional GSS, such as strain instability and reduced productivity in mass-rearing, to be avoided. The sexual competitiveness of released male flies is important for the success of an SIT control program. This paper describes field cage experiments in which the competitiveness of males from a TSS (OX3376B) was compared with that of a conventional GSS (VIENNA-8) and two wild-type strains (TOLIMAN and ARG). When competing for female mates with wild-type males, OX3376B male performance exceeded the minimum acceptable level for a strain intended to be used in SIT. Parallel tests, in which wild-type males competed with either OX3376B or VIENNA-8 males, showed no significant difference between the wild-type males and the males from either sexing strain. When OX3376B males competed directly for mates with VIENNA-8 males, VIENNA-8 slightly outperformed the TSS males. These results suggest that OX3376B in particular, and TSS in general, show sufficiently good mating competitiveness to merit further research into their suitability for eventual use in SIT programs.

Female Lethal RIDL Strains of the Mediterranean Fruit Fly *Ceratitis capitata* and the Mexican Fruit Fly *Anastrepha ludens*

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The Sterile Insect Technique (SIT) has been used extensively for the control of medflies and, on a smaller scale, against other tephritids. Oxitec has developed female-lethal RIDL strains of medfly (*Ceratitis capitata*) and mexfly (*Anastrepha ludens*). The approach has been tested successfully in two tephritid species and we believe it will have wide applicability in other tephritids as well. The strains developed- OX3376B, OX3647Q and OX3864E for Medfly, OX3097B for Mexfly- offer complete female lethality and a heritable fluorescent marker for easy identification. The fluorescent marker on the mexfly line OX3097B has been further tested in collaboration USDA, CPHST (Mission, Tx) and

was found to persist well for at least one week after death in trap fluid (Sierra antifreeze liquid). Line OX3376B when tested in our laboratories has shown excellent penetrance, 100% female lethality in the absence of dietary antidote (tetracycline), good suppressibility of the transgene in the presence of tetracycline and a reasonably good marker. Additional testing of line OX3376B is in progress in Guatemala in collaboration with the US Department of Agriculture and Moscamed. These experiments involve larger numbers and accurate simulation of mass-rearing conditions. Results demonstrate that the technology in general, and some fly strains in particular, have great potential for utilization in SIT-type programs in the future.

Oxitec's RIDL Technology Employs Modern Genetics to Improve the Safety and Cost-Effectiveness of SIT

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The mass release of sterile insects (SIT) is a highly effective area-wide method of pest control. Genetic sexing (male-only release) has proved highly beneficial to medfly SIT programs and is seen as highly desirable for other species. Oxitec has developed a successful genetic system (RIDL) which offers an attractive alternative to conventional sterilization methods, while also completely eliminating females from the release population. Successful female lethal RIDL strains have been developed for two major pests: *Ceratitis capitata* and *Anastrepha ludens*. We consider our system easily transferable to all tephritids, allowing for rapid development of RIDL transgenic sexing strains in all key tephritid pests worldwide. An additional improvement to traditional genetics employed by current SIT programs includes the identification of released individuals with a heritable fluorescent transformation marker. Insect transformation depends on nonautonomous transposable elements as gene vectors. A concern for releasing transgenic insects is the stability of the transgene. Loss of the transgene would result in loss of the strain characteristics important to the program. In this respect, we have further improved our transgenic sexing strains by removing the transposon sequences from inserted transgenes, therefore overcoming potential regulatory concerns regarding stability in SIT programs. The stabilization strategy requires post-integration exposure of the transgene to transposase through the use of a "jumpstarter" strain (a transformed strain that carries the transposase gene) or by re-injection of a transposase source. For an efficient, simple and quick identification of a transposon-free strain, the two transposon ends have been marked with fluorescent protein markers, distinct from the main transformation marker. In summary, by utilizing modern genetics we are offering significant improvements to tephritid SIT programs in four major areas: 100% effective sexing strains; genetic sterilization, easy identification of released individuals and strain stability.

Sesión/Session VII

TIE Procedimientos de Campo. Métodos de Empaque y Liberación de Insectos Estériles/SIT Field Procedures. Packing and Release Methods of Sterile Insects

Avances en los Sistemas de Empaque y Liberación de Moscas de la Fruta Estériles

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La Técnica del Insecto Estéril (TIE) se utiliza en todos los continentes para el control o erradicación de plagas de importancia económica o cuarentenaria, entre otros: gusano barrenador del ganado, gusano rosado, palomilla de la manzana, moscas de la fruta, como parte de un Manejo Integrado de Plagas (MIP).

La TIE no es un simple proceso de recibir pupas y liberar adultos en el campo, implica una serie de eventos que por si solos son ya un proceso industrial, con numerosos y complejos pasos tendientes a lograr un propósito biológico. Cada uno de los pasos de este proceso industrial afecta o pueden afectar la calidad del insecto, por lo que, se han desarrollado Manuales o Protocolos de Control de Calidad y Aseguramiento de Calidad para confirmar que los insectos liberados tienen la máxima capacidad para competir exitosamente en el campo contra los especimenes fértiles.

Investigaciones recientes han identificado que el manejo de las moscas previo a la liberación es crítico y que mediante un manejo especial en los Centros de Empaque, se puede incrementar significativamente la propensión a la copula de las moscas después de su liberación en campo. Durante este periodo se involucran aspectos tales como:

A. Aspectos Abióticos
B. Diseño de las salas

Las salas de Empaque, pero especialmente las de emergencia deben mantener las condiciones ambientales de acuerdo a la biología de las moscas. Los principales parámetros son: Temperatura, humedad, intensidad de luz, ciclo de oscuridad/luz, forma y tipo de alimento. Un parámetro, frecuentemente olvidado, es el de aireación que consiste en garantizar que la calidad del aire sea adecuado para que las moscas respiren aire limpio, lo cual se logra mediante el diseño de un sistema de inyección/extracción que permita intercambios periódicos de aire que eviten la acumulación de gases.

1. Manejo del material biológico

a) Contenedores de empaque

Hay dos parámetros que se deben tomar en cuenta, cuando se define el tipo de contenedor que se va a usar: La densidad de moscas y la superficie disponible para que las moscas extiendan sus alas, de tal manera que se evite la sobre población y el estrés.
Adicionalmente, se ha buscado que haya facilidad para suministrarles agua y alimento a las moscas, en casos de que las condiciones ambientales o cualquier otra causa no permitan la liberación de las moscas en el día previsto. Así como una mejor aireación al tener todos los lados con mallas, este es un nuevo diseño que se esta preparando para utilizarse en el nuevo Centro de Empaque del Programa Moscamed México.

b) Enfriamiento de las moscas

- Cuartos fríos: Se tiene poco o escaso control de la humedad en los cuartos fríos, en la actualidad se maneja una humedad entre el 55 y 65 % sin embargo, frecuentemente se ve a la mosca con exceso de humedad en las maquinas de liberación.
  Este problema se atribuye a que la maquina de liberación no tiene capacidad de quitar la humedad, sin embargo se ha comprobado que cuando se enfría la mosca en los cuartos fríos con una humedad en un rango entre 30 y 40 % de humedad, no se presenta este problema en las maquinas de liberación.

- Maquinas de liberación: El sistema de liberación de adultos enfriados utiliza maquinas con dos principios diferentes de enfriamiento, uno con refrigeración mecánica, es decir que genera temperatura mediante un sistema de enfriamiento instalado en el avión o en la maquina misma de liberación y otro que maneja la temperatura con Dióxido de Carbono congelado mediante ductos e intercambiadores de calor. Ambos sistemas han logrado hacer una liberación más eficiente en términos de control de temperatura y manejo de la humedad en las máquinas de liberación.
  Se investiga en la actualidad el efecto del enfriamiento de las moscas en la cantidad y calidad de producción de feromonas lo cual redunda en el comportamiento y eficiencia de los machos liberados en campo.

c) Aspectos Bióticos

- Hormonas: La Hormona Juvenil (Precocene) acelera la madurez de los ovarios en mosca del mediterráneo, aunque reduce la atracción de machos a hembras, sin embargo, la hormona juvenil (Metoprene o fenoxycarb) en *Anastrepha spp* es crucial en la regulación del desarrollo de la competencia reproductiva y la feromona de llamado, al acelerar la copula en 4-5 días antes que los machos no tratados. El Metoprene ya se esta utilizando con *Anastrepha ludens* en Nuevo León y San Luis Potosí, México.

- Nutrientes: Se ha demostrado que el alimento que contiene azúcar y proteína incrementan el llamado, la competitividad sexual y reproducción y que la cantidad de feromona producida es 10 veces menos en los machos alimentados sólo con azúcar. Cuando se mezclan el uso de hormonas y la adición de proteína en el alimento de las moscas se incrementa sustantivamente su comportamiento sexual y reproductivo.

- Semi-Químicos: El metil-eugenol incrementa significativamente la copula en Bactrocera dorsalis, mientras que el aceite de jengibre lo hace con Ceratitis capitata. Se ha implementado el uso del aceite de jengibre en los centros de empaque de Florida, California, México y Guatemala.

2. Métodos de Liberación

La liberación aérea ha demostrado ser más eficiente en cuanto a distribución homogénea de las moscas comparada con la liberación terrestre. En cuanto a la liberación aérea hay diferentes tipos de aviones y maquinas de liberación que se usan de acuerdo al tipo de terreno y la extensión de superficie que se cubre, por lo que se pueden usar helicópteros para terrenos montañosos y aviones monomotores o bimotores con maquinas de 2.5 millones de moscas por vuelo hasta maquinas de 20 a 25 millones que pueden hacer vuelos de 60 a 75 millones de moscas. Los aviones que utilizan sistemas de de aeronavegación con geoposicionadores han demostrado ser mas eficientes en la distribución de las moscas.

a) Control de Calidad o Aseguramiento de Calidad

Hay manuales detallados para la evaluación de la calidad de las moscas de la fruta conocido como manual de calidad del producto y utilizado en todos los centros de empaque del mundo, sin embargo en México se desarrolló un sistema de aseguramiento de calidad para evaluar el proceso de liberación de *Anastrepha* spp. Este sistema llamado MACX es una herramienta que ayuda a evaluar cada paso del proceso desde que sale de la Planta de Producción hasta que es liberado en campo y que junto con las maquinas de liberación Mubarqui, esta diseñado para dar seguimiento a la liberación de adulto enfriado, transmitiendo en tiempo real la información de calidad de la liberación tales como volumen, temperatura y humedad, además de velocidad, altura y posición cada segundo.

Cada día es más frecuente el uso de la TIE como parte de un Manejo Integrado de Plagas en áreas extensas que inician a un nivel de pequeña escala, pero cuando las condiciones socio políticas lo permiten, se transforman en acciones de mayor nivel.

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Resúmenes de Posters Presentados/Abstracts of Presented Posters

Field Dispersal and Survival of Sterile Medfly Males *tsl* Strain, Aromatically Treated with Ginger Root Oil

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Recent studies have shown that aromatherapy of sterile Medfly males (*Ceratitis capitata)* with ginger root oil (GRO) has improved mating performance, and increased SIT efficiency significantly. However, this efficiency is also dependent on their dispersal ability and survival under field conditions. Thus, the objective of this work was to evaluate the dispersal and survival of sterile Medfly males treated and untreated with GRO under open field conditions. Experiments were carried out in Petrolina-PE, Northeast of Brazil, from May/2006 to December/2007. The *tsl* strain used was Vienna 8 from the Medfly mass-rearing facility located in Juazeiro-BA, Brazil. Pupae were divided into 2 lots (red and blue DayGlo dye color), and irradiated (95Gy from a Co^{60} gamma source) 24 to 48 h before emergence. When flies were 4 days old, either lot red or blue was exposed to 1.5 ml of GRO for 20 hours in a closed room ($27m^3$). Early the next day, ca.10 thousand of each color of dyed fly, exposed and unexposed to GRO, were released at the center of a 25 ha grape orchard. To monitor flies, a grid of 48 Jackson traps baited with trimedlure were placed in concentric circles, at various distances (25-250 m), around the release point. We found no statistical difference in the dispersal behavior and survival between sterile males exposed or not exposed to GRO. More than 60% of sterile males, treated and untreated, were recovered 25 m from the release point, ca. 20% at 50 m, and a ca, 5% in traps 100 m from the released point. Around 90% of the sterile males, exposed and unexposed to GRO, where recovered up to 5 days after release, while less than 1% were recovered 11 days after release.

Evaluación del Sistema "Adulto Frío" en el Empaque para Liberación de *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), Parasitoide de Moscas de la Fruta

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Se evaluó la técnica del adulto frío en el empaque para liberación de *Dichasmimoprha longicaudata* empleando tres dispositivos de empaque con diferentes densidades. La supervivencia, fecundidad y habilidad de vuelo fueron los parámetros indicativos usados en las evaluaciones, así como el porcentaje de estructuras dañadas (antenas, alas, patas) en parasitoides de cada tratamiento. Como

paso inicial se determinó que los parasitoides sometidos a 3° C durante 45 min, permanecían aletargados (y por consecuencia se facilitaba su manejo durante el empaque) sin que se presentaran efectos significativos en los parámetros evaluados. Se observó que la supervivencia, fecundidad, habilidad de vuelo y porcentajes de estructuras dañadas disminuyeron conforme se utilizaron densidades menores. Condiciones de mayor aeración (cajas PARC modificadas y cribas en torres) mostraron los mejores resultados aunque todavía menores que el testigo. El mayor porcentaje de daño de los parasitoides empacados se presentó en las antenas (~ 70%), por lo que se evaluó su capacidad de búsqueda en comparación con parasitoides sin daño (testigo) y dañados artificialmente (pero sin enfriarse). Los resultados mostraron que los parasitoides enfriados fueron los mas lentos en responder ante la presencia de frutos infestados por moscas de la fruta, pero con el paso del tiempo mostraron una recuperación paulatina y consistente, sin que se presentaran diferencias significativas entre tratamientos a los 60 min de observación. Estos datos sugieren la posibilidad de utilizar como densidad de empaque 20,000 y 16,500 pupas por caja PARC modificada y Cribas en torres respectivamente, en aquellos programas de control biológico por aumento que se desarrollen a nivel regional.

Developing and Evaluation of the "Mexico Type" Emergence Tower for Holding *Anastrepha* spp. During the Packing and Releasing Process of Sterile Fruit Flies in Mexico"

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The environmental conditions into the pre-release emergence rooms play an important role for the quality of the sterile insects. This environment includes the type and size of the fly holding container and the specific ambient conditions of the holding room (IAEA, 2004). The environmental conditions in the fly holding room are also potentially critical; parameters as light, temperature, humidity, and barometric pressure may all play important roles in conditioning the flies for their vital role in the field: surviving and attracting, courting, and copulating with wild females. In 2002, the Florida Preventative Release Program against medfly began using a new system, the Tower Eclosion (TE) system, for emergence and feeding of adults prior to field release. This system offers several advantages, saving space, labor costs, reduce disposal and supply costs. Considering this results in *Ceratitis capitata*, the National Campaign against Fruit Flies in Mexico, developed a new emergence system for holding *Anastrepha* spp. during the packing and release process of sterile fruit flies, "Mexico Type Emergence Tower". The "Mexico Type Emergence Tower" was evaluated using three different sterile fly densities of *Anastrepha ludens* and we found no statistical differences among the densities evaluated and the control treatment. The sexual behavior of *Anastrepha ludens* sterile flies when they were seven days old treated with Juvenile Hormone and packed in Mexico Type Emergence Tower, showed statistically better behavior compared with sterile flies packed in Worley Tower, in Montemorelos, Nuevo Leon, Mexico. The result obtained presents a new alternative that can be considered for its implementation. This system can enhance the quality of the flies considering that the insects are holding in relaxed environment and the impact of stress for relative humidity changes can be reduced.

Development of a New Sterile Insect Aerial Release Machine

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A new aerial release machine (Mubarqui Release Machine.2) has been built and tested designed to release 6.5 million sterile *anastrepha ludens* per release. The MRM.2 was designed to be economically feasible and autonomous (it can be transported in single engine Cessna 206, Piper Cherokee, Maule m7). The main body of the MRM.2 is built in stainless steel and it is fully isolated. Flies are kept in a specially designed cooled chamber. The container can be filled with 6,500,000 sterile flies. It has two electrical systems to generate air cooling: one in 12 or 24 volts D.C. and the other on 110 volts A.C. Two options are used because power in aircraft is 12 or 24 VDC, and in the rearing facility is 110 VAC. The MRM.2 has a higher capacity container and lower volume design, Air is circulated by electrical fans in specially designed conductions that have been included in the main body. This system eliminates water condensation and allows achieving a uniform inside air temperature. The desired temperature is maintained by an electronic control. Flies are not dosed using an auger but a flat belt conveyor, avoiding mechanized damage, and are released by an air flow produced by the aerodynamically suction from the external air flow. Sterile flies are directed to the orchards. Field tests have demonstrated that the MRM.2 is capable of maintaining the relative humidity and temperature in the requested conditions during 4 hours and the flies was kept in optimal conditions, thermostat maintain the temperature in 1 to 2 C degree with less than 1% variation. Different controls are used to calibrate the sterile male adults' density, varying 2 parameters, and belt speed and caliper gate. The MRM.2 temperatures and relative humidity were tested. Thereafter, a field test was performed to assess the efficiency of the MRM.2.

Development of a New Software Runing on Internet for Sterile Fruit Flies Release

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A new software PROMOMED has been designed and tested to assure all involved actions in integrated management and release of sterile insects. The software was designed to be a feasible and friendly tool, which can be rune in any PC with internet by all involved actors: from production facility to aerial release on field. The characteristics of this software are:

- Web System compatible with any operating system and any explorer of Internet.
- It is an interactive system that tells on a data base adapted the needs of the program.
- Ease of use. Since for each area it shows a specific menu. This avoids that the user loses itself between menus that are not going to use.
- Easy capture, important point since the system is administering the information. This means that by each area the minimum information is captured and this is used for other areas in different processes, with this is avoided the capture duplicity
- The system includes the areas of quality control, programming, release, recaptures, statistic and geocontrol. It also has a library in which the system keeps the information from track of flight in different formats and polygons.

- Page Web Promomed (macx system). Is a program to assure quality of the different processes, which include since pupa is received to adult aerial release. This software also integrate the information generated from aerial release, terrestrial release, recaptures, distribution percent, trapping net, FTD, graphs of FTD, sterile-wild rate, file downloading flight track, fast consultations of plague movement, detection of buds, population dynamics. Thus also capacity of downloading of the realized consultations and compatibility of all these files with the system google earth. www.promomed.org.mx .

Incorporating Juvenile Hormone into the Mubarqui® Adult Diet to Enhance the SIT Effectiveness in the Mexico National Campaign against Fruit Flies

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The evaluation was realized in two sites, the first one was at the sterile Mexican fruit fly packing and release center (CEAF) of Montemorelos, N. L., Mexico, were one batch per shipment received from Chiapas were randomly selected and two types of emergence towers (Worley and Mexico type tower) were used for the test. The flies were packed and Mubarqui adult diet with methoprene was provided as adult food. The same type of diet without methoprene was used as control treatment in both types of towers. The second site was in Sinaloa, Mexico were sterile Mexican fruit flies were packed in "Mexico type emergence towers" and Mubarqui adult with methoprene was provided as adult food and compared with diet without methoprene. The sexual behavior in both sites was recorded. In order to evaluate the incorporation of Juvenile Hormones (JH) at massive levels, we select two pilot areas (blocks) of approximately 3,000 has. in Montemorelos, Nuevo León, Mexico and 8 millions of JH treated flies per week were released; sterile flies without JH were released in the second block used as control treatment. We realized 16 replications and trapping monitoring and fruit sampling was recorded. In Montemorelos, Nuevo Leon, Mexico, the 60% of *Anastrepha ludens* holding in Mexico type tower shows sexual behavior after two days of released on field against 20% of control. In another hand, 20% of *A. ludens* holding in Worley tower shows sexual behavior after two days of released on field against 18% of control. The treatments with different letter showed statistical differences. The 100% of *A. ludens* treated with Juvenile Hormone shows sexual behavior after six days old insects at the fruit fly packing and release center of Sinaloa, Mexico, while the control shows sexual behavior until 11 days old insects.

Flyagra for the SIT: Can Melon Flies Get Juiced up, Too?

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Chemotherapy to improve the sterile insect technique has been successfully applied on a mass scale worldwide for the Mediterranean fruit fly, *Ceratitis capitata*. In this study, we have examined the possibility of increasing the sterile male efficiency of the melon fly, *Bactrocera cucurbitae*, through exposure of sterile males to one of several melon fly attractants- melolure, cuelure, and zingerone. Reproductively mature and marked (dyed) sterile males of the melon fly genetic sexing strain were exposed to one of several male lures. For the liquid attractants, melolure and cuelure, we exposed each to males in a cubical cage. For zingerone, pure crystal was exposed to males in a cubical cage and a control cage was also maintained without exposure to any treatment. Wild flies were raised on zucchini fruit as larvae and maintained as adults in cubical cages until ca. 35-45 days old and reproductively mature for field testing. On the test day, an equal number of wild and sterile males (treated or control) were released into several field cages. Then, wild females were released into the cage and mating activity commenced. Pairs were collected for several hours until mating activity terminated. The male type, lab or wild, in each pair was determined by examination of the ptilinum and the data recorded. The proportion of sterile matings in each treatment cage compared to the control was calculated. Four or five replications of each treatment were performed. Results indicated that exposure to melolure and cuelure raised the mating competitiveness of sterile males ca. 25% ($p < 0.01$), while for zingerone it raised it ca. 20% ($p< 0.05$). Additional tests are planned to evaluate zingerone exposed as a liquid, and further to know whether the effects of lure exposure can last longer than a single day.

A 48-H Pre-Release Protein Diet is Sufficient to Increase Sexual Performance of Male Queensland Fruit Flies *Bactrocera tryoni* (Diptera: Tephritidae)

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Recent studies have shown that continuous access to a protein source (yeast hydrolysate) can greatly enhance the sexual performance of male Queensland fruit flies (*Bactrocera tryoni* ; 'Q-flies'). However, in Sterile Insect Technique programmes used to eradicate or suppress wild populations, mass-reared Q-flies are typically fed only sucrose and water for up to 2 days before release. Here we investigated whether adding a protein source to the diet of male Q-flies for a 24- or 48-h window after emergence and then removing it is sufficient to enhance mating probability, latency to mate, copula duration, probability of sperm storage, number of sperm stored, female remating tendency and longevity of male Q-flies. Protein-fed males were more likely to mate than males fed only sucrose, especially when young. Protein-fed males also had shorter mating latencies and longer copulations than protein-deprived males. Females mated by protein-fed males were more likely to store sperm, stored more sperm and were less likely to remate than were females mated by protein deprived males. Females were also less likely to remate if their first mate had been large. Overall, providing male Q-flies access to a protein source for a 24- or 48-h window early on in their adult life was sufficient to greatly enhance all assessed. Measures of performance. Although 24-h access was sufficient for a notable enhancement,further benefits were evident in males provided 48-h access. Results are encouraging for Sterile Insect Technique programs used to eradicate or suppress wild Q-fly populations.

Optimising Irradiation Procedures for Sterilization of Queensland Fruit Flies.

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The Sterile Insect Technique (SIT) is an important pest management program that is used for containment and eradication of Queensland fruit fly (Q-fly) outbreaks. In SIT, flies are mass reared, rendered reproductively sterile by gamma radiation, and then released. Sterile males mate with wild females, preventing the production of viable offspring and instigating a population crash. The present study is an important part of calibrating, validating and improving irradiation methods used in the current Q-fly SIT program. We used routine IAEA/USDA/FAO quality control tests including flight ability, longevity, sterility and mating competitiveness to assess the impact of irradiation dose rate and target total dose on the product quality of mass reared Q-flies used for SIT. We found a strong relationship between dose rate and over-shooting errors in target dose (currently 70-75 Gy) that resulted in significant reductions in longevity of irradiated flies. Further investigations of target irradiation dose (60, 65, 70, 75 and 80 Gy) revealed reductions in longevity and competitiveness as dose increased, while sterility induction remained adequate even at doses as low as 60 Gy. The impact of dose rate and target sterilizing dose on the quality of mass reared Q-flies, and the potential for revised protocols to yield flies of superior quality, is discussed.

¿Afectan las Hembras Estériles el Desempeño Sexual de los Machos Estériles de *Anastrepha ludens* (Díptera: Tephritidae)?

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La Técnica del Insecto Estéril (TIE) es un método de control exitoso en los cinco continentes para suprimir y erradicar un gran número de especies de insectos plagas. Implica la cría, esterilización y liberación de un gran número de machos estériles para aparearse con hembras silvestre y así reducir la población silvestre, sin embargo, estos procesos producen una alteración significativa que incide negativamente en el comportamiento sexual, por lo que constantemente se buscan alternativas de mejora. En años recientes, el desarrollo de cepas modificadas genéticamente permitió la separación de los sexos. En *Ceratitis capitata* se observó que las cepas de solamente machos mejoran la inducción de esterilidad en campo comparadas con cepas bisexuales. Sin embargo, sólo un número pequeño de pruebas de campo demuestran el potencial de liberar solamente machos. En la mosca Mexicana de la fruta *Anastrepha ludens* (Loew), actualmente las liberaciones se realizan por medio de cepas bisexuales. En un esfuerzo por medir el grado de impacto que puede presentar la liberación de solo machos de *A. ludens*, se evaluó bajo condiciones de jaula de campo, el comportamiento de apareamiento y la inducción de esterilidad de machos de cría estériles con insectos silvestres bajo diferentes densidades de liberación en presencia y ausencia de hembras estériles. Este estudio indicó que la presencia de hembras estériles afecta significativamente el desempeño sexual de los machos estériles con hembras silvestres. Se observó que la cantidad de machos estériles liberados es proporcional a la esterilidad inducida en las poblaciones silvestres. Estos resultados indican que la posibilidad de liberar sólo machos estériles, puede mejorar la eficiencia de la TIE para el control de la Mosca Mexicana de la fruta *A. ludens*.

Importancia del Tamaño del Macho en la Recópula de *Anastrepha ludens* (Díptera: Tephritidae)

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En este estudio evaluamos el efecto del tamaño del adulto en la elección de pareja, frecuencia de cópula e inducción del periodo refractario de la hembra silvestre de la mosca Mexicana de la fruta *Anastrepha ludens* frente a machos silvestres y estériles de laboratorio en competencia bajo condiciones de laboratorio. En la competencia entre machos pequeños (pupa con diámetro de 2.15 a 2.45 mm) la hembra silvestre eligió para su primer apareamiento el 55.17 % a machos estériles y el 68.42 % a machos silvestres, mientras que con machos grande (pupa con diámetro de 2.60 a 2.90 mm) fue del 50% para ambos. La frecuencia de apareamientos de las hembras con machos pequeños fue de 2.28 cópulas por hembra, mientras que con macho grande fue de 1.80. En la competencia entre machos grandes (2.60-2.90 mm de diámetro) y pequeños (2.15-2.45 mm de diámetro) de laboratorio fértiles, la hembra silvestre eligió para su primer apareamiento el 20% a machos pequeños y el 80% a machos grandes. Finalmente, el periodo refractario inducido por machos pequeños fue significativamente mayor con machos silvestres que con machos estériles, con machos grandes no se presentó diferencia significativa. En conclusión los resultados de este estudio indican que la producción de pupas grandes dentro de una cría es importante dentro de los programas de control ya que los adultos grandes al competir con machos silvestres por la elección de las hembras tendrán mayor oportunidad de ser elegidos, además reducirán la posibilidad de que la hembra vuelva a aparearse, disminuyendo la posibilidad de que la hembra cambie su gravidez de infertilidad a fertilidad.

Development of Radio-Controlled Aircraft for Release Sterile Flies

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The Medfly facility is located in Sao Francisco Valley, in northeast of Brazil. The Valley has a semi-arid climate, quite flat topography with elevation around 370 meters above sea level, and around 80,000 hectares of fruit crops divided in irrigation districts reasonably isolated one from another. A fruit fly suppression program employing SIT, cultural and chemical control is being carrying out targeting medfly and *Anastrepha* species. The Valley has a perfect landscape and agricultural land for the use of area-wide concept and control by SIT. Aerial releases are one of the most important methods used in SIT programs. Fixed wing airplanes with manual or releasing machine have been used in many SIT programs around the world. Radio-controlled aircrafts (RC plane) for releasing chilled medfly adults were designed and tested as an alternative for regular airplanes. The aircraft is 2.00 meters long, 3.60 m wingspan equipped with a 50 cc gas engine. Three prototypes were tested and the latest one has 20 liters of loading capacity that can hold around 1 million flies per flight. The RC plane is easy to operate in flat terrain and its flight is limited by the operator visual contact. Usually the operator can fly a RC plane in an area of 50 hectares. The present model needs a runway 20 meters long for takeoff and 30 to 40 meters for landing. The RC plane flight altitude ranges from 300 to 400 feet, releasing flies just above the target orchards. The average speed is 50 to 60 km per hour during releasing procedures. The cost to

release 1 million flies is US$ 85. RC plane under the Sao Francisco Valley is a good option, at low cost and very reliable operation, releasing the sterile flies at low altitude and low speed, causing less damage to the flies.

Esterilidad Inducida en Machos y Hembras de *Anastrepha fraterculus* (Wiedemann) - Perú

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Se efectuó una serie de experimentos con el fin de determinar niveles de esterilidad en machos y hembras aplicando dosis de radiación gamma de 30, 40, 50, 60, 70 y 80 Gy hasta determinar la dosis apropiada que asegure completa esterilidad, comprobándola con el seguimiento de fertilidad de sus progenies. Se irradiaron pupas de 60, 48 y 24 horas antes de la emergencia de la colonia de crianza artificial de Perú (origen Piura 2000, Lima 2002 e Ica 2004), cruzando 25 parejas de adultos irradiados con no irradiados del sexo opuesto, colocados en envases plásticos con panel de oviposición, agua y alimento. El fotoperiodo fue de 10 horas luz y 14 oscuridad a 26.0 ºC y 60% HR. Los huevos colectados se alinearon en superficies humedecidas y se colocaron a 28 ºC y 80% HR, a los dos días se transfirieron a dieta larvaria. Se realizaron lecturas de huevos eclosionados y los adultos obtenidos como resultado se cruzaron con moscas no irradiadas del sexo opuesto midiendo la fertilidad de la progenie reproduciéndola hasta estado adulto. Se logró determinar que a partir de 50 Gy se obtiene esterilidad completa en hembras expuestas en estado de pupa a 60, 48 y 24 horas antes de la emergencia. Asimismo se determinó que 70 Gy es la dosis para inducir esterilidad completa en machos de pupas irradiadas a 60 y 48 horas antes de la emergencia, así como la dosis 80 Gy en pupas irradiadas a 24 horas antes de la emergencia, ya que las progenies de estos individuos no son capaces de progresar a la siguiente generación, dosis recomendables a aplicar para realizar las pruebas de competitividad de cópula y su posterior aplicación e implementación de la TIE de esta especie de mosca de la fruta en el Perú.

Aerial Release of Sterile Flies Using Radio-Controlled Aircraft

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Sterile Mediterranean fruit flies, *Ceratitis capitara*, were realased over commercial 9.2 hectares mango field using a radio-controlled aircraft 2.00 meters long, 3.60 m wingspan equipped with a 50 cc gas engine in Sao Francisco Valley, State of Pernambuco, Brazil. The objective was to evaluate the use of RC aircraft as releasing method and determine the distribution pattern, recapture rate and survivorship in the field. Flies 3-4 days old were knock down at -4^{o}C for 15 minutes in the laboratory and brought to the release point in ice cooler with dry ice 20 min driving. Three releases of approximately 60,000 sterile males were carried out at 50 to 100 meters altitude and 50-60 km speed in one single line through middle of the mango orchard with green fruit. After releases, 64 Jackson traps baited with trimedlure were installed in days 0, 1, 2, 4, 8 and 16 and exposed for 2, 4, 6, 8, 8 and 8 hours, respectively. Spatial and temporal dispersal pattern of the released males were

according decay models. The dispersion was not uniform and varied in each release. Recapture rate ranged from 0.83 to 2.28%. The highest capture in day one after release and flies were found until the 16th day. The data suggest that RC aircraft should be a good inexpensive alternative for aerial release when compare with conventional airplane, take in consideration, release altitude, aircraft speed during releases and flight precision over the target area.

Low-Cost Isotope Marking Method for the Mediterranean Fruit Fly *Ceratitis capitata* Wied (Diptera: Tephritidae)

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A simple method of marking of Mediterranean fruit flies *Ceratitis capitata* (Wiedemann) using stable isotopes is described. This species is an economically important pest in agriculture and has a major impact on agricultural trade. It is a target species of many successful area-wide integrated pest management (AW-IPM) programmes using the Sterile Insect Technique (SIT). Programme monitoring in the field relies on being able to accurately differentiate released sterile insects from wild insects so that estimates can be made of the ratio of sterile males to wild males. Typically, released flies are marked with fluorescent dust 100 % reliable. All known species of fruit fly feed on C3 plants in the wild which have a carbon isotope signature of around -28‰ on an internationally recognised scale. However, almost all mass rearing facilities use cane sugar in the larval and adult diet which is a C4 sugar sources (with a signal of around -11‰) and this could provide a an easy signature to differentiate released flies from wild flies. To test this approach, samples of flies from several operational field programmes from the field and the facility were analysed. It was clearly demonstrated that using C4 sugar in the larval rearing diet was an effective and economic way of intrinsically labelling Mediterranean fruit flies and it was possible to distinguish mass reared from wild populations with greater than 95% confidence. The C4 marker was detectable and distinguishable from wild populations up to twelve days after "release". This technique could be extending to any other SIT programs for other fruit flies species that use similar rearing protocol than Medfly.

Male Mating Competitiveness and Sterility Induction into Cohorts of Wild *Anastrepha ludens* (Diptera: Tephritidae) by Mass Reared Flies Irradiated at Different Doses

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In several tephritid species females are more radiosensitive than males, in order to achieve 100% sterility of both males and females in bisexual strains released as part of Sterile Insect Technique programes, high doses of irradiation have to be applied. Substantially lower doses are sufficient to

completely suppress egg production in irradiated females and still achieve levels of sterility above 95% in males. Males irradiated at low doses may be more competitive in terms of mating success than males irradiated at high doses, compensating for releases of some fertile individuals. Here we compared male mating success and sterility induction of bisexual cohorts of *Anastrepha ludens* irradiated at a high (regular dose applied before release) and a low dose (dose applied to achieve egg production suppression in females) into a cohort of wild field collected Mexican Fruit flies. In field cages, males irradiated at high doses were accepted lower numbers than wild males by wild females, while males irradiated at low doses were accepted in similar numbers than wild males. Egg hatch of eggs laid by wild females released in cages with a) wild males, b) wild males and flies irradiated at low doses, and c) wild males and flies irradiated at high doses was highest for females recovered from cages with wild males (a), followed by cages with wild males and flies irradiated at high doses and was the lowest for cages with wild males and flies irradiated at low doses (b).

Desarrollo de una Dieta Larvaria a Base de Tempe de Garbanzo (*Cicer arietinum* L.) Como Fuente de Proteína Para A*nastrepha ludens* y *Anastrepha obliqua* (Diptera: Tephritidae)

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La Planta Moscafrut produce semanalmente 220 y 40 millones *Anastrepha ludens* y *A. obliqua* respectivamente, utilizando dietas larvarias preparadas con levadura tipo torula como principal fuente de proteína y vitaminas, con las cuales se obtienen larvas con peso menor en comparación con larvas obtenidas de frutos. Por ello se buscan fuentes alternativas ricas en proteína como es el caso del "tempe", el cual es producido mediante fermentación en estado sólido de la harina de semillas de leguminosas por el hongo *Rhizopus oligosporus*. Con base en el contenido proteínico del tempe se formularon nuevas dietas larvarias para *A. ludens* y *A. obliqua*. La fuente de proteína en la dieta control fue la mezcla de harina de maíz+levadura torula, en las dietas de prueba el tempe sustituyó a cada uno, quedando los tratamientos como tempe+levadura torula y tempe+harina de maíz. Para *A. ludens* no se observó diferencia significativa al sustituir la harina de maíz por el tempe, pero al sustituir la levadura por el tempe los parámetros de producción disminuyeron significativamente. La transformación de huevo a larva disminuyó de 93.3 a 83.8%, el peso larvario de 32.4 a 25.9 mg, la pupación 24 horas de 98.1 a 97.9 %, el peso de pupa de 23.8 a 18.3 mg y la emergencia de adultos de 98.2 a 97.3 %. Para *A. obliqua* los parámetros analizados no presentaron diferencia significativa entre las tres dietas probadas, con valores de transformación de huevo a larva de 88.9%, peso larvario de 18.6 mg, pupación 24 horas de 98.5 %, peso de pupa de 15.8 mg, emergencia de adultos de 84.7 % y 79.3 % de voladoras. Los resultados indican que el tempe de garbanzo es una buena fuente de proteína alternativa como sustituto de la levadura en la dieta de *A. obliqua* y como sustituto de la harina de maíz para *A. ludens.*

Sesión/Session VIII

Control Químico, Estaciones Cebo y Tratamientos Cuarentenarios/Chemical Control, Bait Stations and Quarantine Treatments

Uso de la Irradiación para Propósitos Fitosanitarios

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Panorama fitosanitario

En el comercio internacional de productos agrícolas, la mayoría de los gobiernos han establecido programas de protección fitosanitaria con el fin de evitar posibles infestaciones de plagas ajenas, que son complejos en su naturaleza, exigiendo cuarentenas parciales o totales, o en su caso, la aplicación de tratamientos dirigidos a la eliminación de esas plagas específicas.

En México, el esfuerzo principal de las entidades gubernamentales encargadas de la sanidad vegetal se han enfocado en combatir la presencia de la Mosca Mexicana de la Fruta (*Anastrepha ludens* (Loew)), así como prevenir la invasión de la Mosca Mediterránea de la Fruta (*Ceratitis capitata* (Wiedemann)) de Guatemala hacia nuestro país. Estos trabajos también han tenido un impacto favorable en prevenir las apariciones en los Estados Unidos de este tipo de plagas, siendo un requisito para aceptar la importación de frutas mexicanas en ese país.

Para este último objetivo, el Departamento de Agricultura de los Estados Unidos (USDA) colabora de manera cercana con la Secretaría de Agricultura de México (SAGARPA) y a través de la Dirección General de Sanidad Vegetal (DGSV), se ha establecido un cordón fitosanitario, para proteger la región norte del país, exigiendo tratamientos[1] y certificación para productos hospederos de la mosca de la fruta que ingresan a esas zonas; dicho cordón de seguridad que va desde Nayarit hasta Veracruz, cruzando toda la República Mexicana, de manera que aún para la movilización nacional de estos productos, son requeridos dichos tratamientos (Figura 1).

La Organización de las Naciones Unidas (ONU), a través de organismos multinacionales como la FAO, el OIEA y otras agencias nacionales e internacionales, han estado promoviendo el tratamiento de irradiación gamma con fines fitosanitarios, que ya fue aprobado por USDA desde el 23 de octubre de 2002; desde entonces, las normas vigentes han sido ampliadas constantemente para ser capaces de alcanzar una mejor utilización de esta tecnología. El proceso ha sido acelerado por la necesidad identificada de la comunidad internacional de eliminar los principales fumigantes químicos, como el bromuro de metilo, que contribuye de forma substancial a los cambios climatológicos a través del efecto invernadero y el daño a la capa de ozono del planeta.

La irradiación gamma para propósitos fitosanitarios

La irradiación de frutas frescas hospederas de mosca mexicana de la fruta es una alternativa de tratamiento eficaz, por la capacidad de penetración que tiene la radiación gamma en la fruta, lo que permite neutralizar esa plaga y algunas otras. La tecnología de irradiación gamma ofrecida por BENEBION tiene adicionalmente el beneficio de tratar la fruta en tarimas industriales, lo que evita la manipulación excesiva de la misma, reduce mermas del producto y el riesgo de reinfestación. Esta tecnología está aprobada en México como una alternativa de tratamiento fitosanitario[2] (Figura 2).

[1] Ver Norma Fitosanitaria NOM-075-FITO-1997
[2] Ver Norma Mexicana NOM-022-FITO-1995

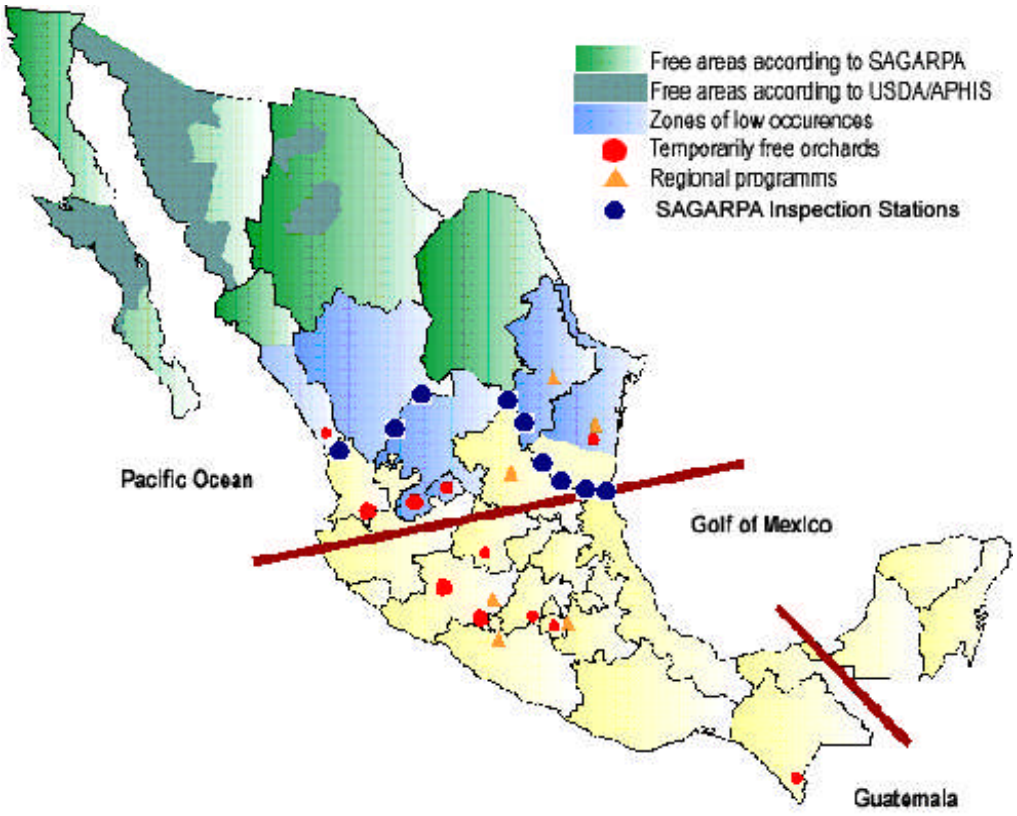

Figura. 1.- Ubicación del cordón fitosanitario en la República Mexicana.

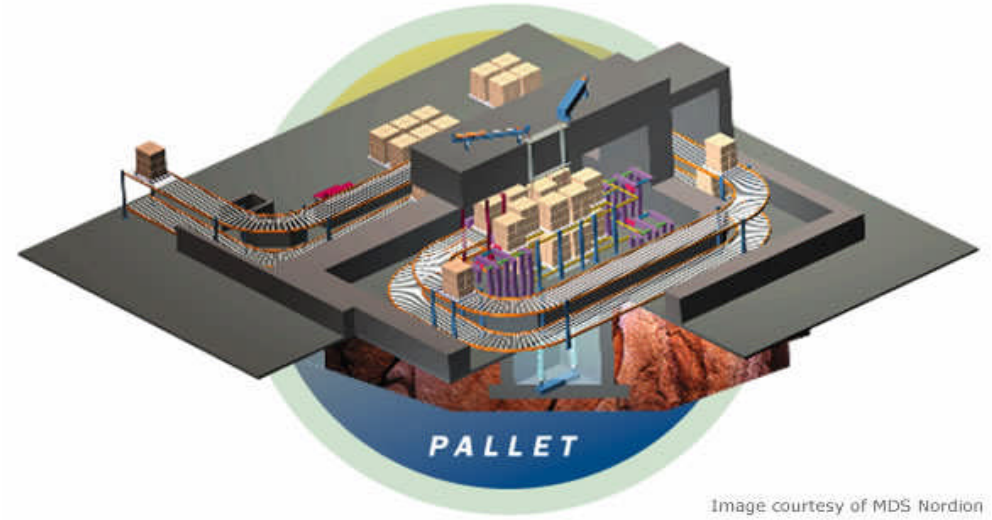

Figura 2.- Esquema de irradiador gamma de tarimas industriales[3] que BENEBION esta construyendo en Matehuala, SLP

[3] (Private) Licensing Information Package for the IR-224/225 Two Pass Parallel Row Pallet Irradiators, PHYTOSAN, MEX by MDS Nordion

Requerimientos de empaque

Los gobiernos de México y los Estados Unidos han firmado un Plan de Trabajo Operacional[4] para la exportación al vecino país de algunas frutas frescas hospederas de mosca de la fruta, donde se especifican los requerimientos de empaque y las características que deben cubrir las empacadoras y las instalaciones de irradiación para tratar dichas frutas frescas, que deberán estar empacadas en cajas de cartón a prueba de insectos, para prevenir la oviposición en la fruta de las cajas y apiladas en tarimas industriales.

La tecnología de la irradiación con fines fitosanitarios para frutas frescas presenta ventajas importantes para los productores, los exportadores, las agencias gubernamentales y los consumidores, así como para el ambiente, en comparación con los tratamientos convencionales en los que se utiliza el bromuro de metilo o algunos tratamientos térmicos que se usan actualmente. Dichas ventajas incluyen:

- Proceso industrial altamente reproducible, lo que garantiza una erradicación exitosa de una gran gama de plagas de insectos con alta eficacia.
- Muchos tipos de fruta que no pueden ser exportados actualmente, por no ser factibles de tratar por métodos convencionales, podrán tratarse a través de la irradiación con resultados satisfactorios y sin daños a los productos.
- Tiempo de procesado rápido (del orden de una hora y media por embarque).
- Procesamiento completo en tarimas comerciales y sin manipulación adicional.
- Costos bajos en comparación con procesos alternativos.
- Un nivel elevado de seguridad alimenticia microbiana.
- Sin impactos ambientales.
- Potencial de aumentar la vida de anaquel del producto.
- Mejora la inocuidad del producto.

[4] IRRADIATION OPERATIONAL WORK PLAN Between México and United States of America. October 2007

Resúmenes de Posters Presentados/Abstracts of Presented Posters

Evaluación del Lufenuron como Quimioesterilizante contra Moscas de la Fruta del Género *Anastrepha* Schiner (Diptera: Tephritidae)

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El lufenurón es un regulador de crecimiento con eficacia demostrada como quimioesterilizante en el control de la mosca del Mediterráneo *Ceratitis capitata* (Wied.). Los quimioesterilizantes pueden constituir una alternativa asequible en el caso de especies de moscas donde la implementación de la Técnica del Insecto Estéril no sea una alternativa viable; por ejemplo *Anastrepha striata* Schiner y *A. serpentina* (Wied.), especies de importancia económica en diferentes países de América Latina. En este trabajo se evaluó la susceptibilidad al lufenuron de cuatro especies del género *Anastrepha* Schinner de importancia en México. Los adultos maduros se colocaron en jaulas de 30x30x30 cm con paredes de malla mosquitero y fueron alimentados por 24 h con lufenuron (100, 500, 1,000, 5,000. 10,000 y 30,000 ppm, mezclado con proteína y azúcar (3:1). Cada tercer día se colocaron hospederos artificiales por 24 h; y durante 11 días se cuantificó la eclosión larvaria. También se realizaron pruebas cruzadas: 1) hembras tratadas/machos sanos, 2) hembras sanas/machos tratados y 5) hembras sanas/machos tratados. Todas concentraciones de lufenuron redujeron significativamente la fertilidad en *A. ludens* hasta el día 11, pero posteriormente los valores se incrementaron aunque sin alcanzar los registros del testigo. En *A. obliqua* la reducción fue drástica durante todo el tiempo de estudio. Con *A. serpentina* el impacto se observó en el primer día y posteriormente hubo una recuperación paulatina pero constante. En el caso de *A. striata* el efecto fue significativo, excepto para la dosis de 100 ppm. Las pruebas cruzadas indicaron que en *A. ludens*, *A. obliqua* y *A. serpentina* la esterilidad no se transmitió de machos a hembras ya que los valores de eclosión de huevecillo fueron similares a los obtenidos con individuos sanos. Solamente en el caso de *A. striata* la cruza de machos tratados con hembras sanas redujo significativamente la fertilidad del huevecillo.

Novel Bait Stations for Attract-and-Kill of Pestiferous Fruit Flies (Tephritidae) in Hawaii

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Behavioral approaches to the management of fruit flies are an excellent alternative to the conventional application of insecticides. Bait stations represent one type of attract-and-kill approach to fruit fly management. We developed a novel rain-fast bait station that takes advantage

of the flies need for shelter and their strong response to yellow color while protecting GF-120 Fruit Fly Bait against rainfall. Here we describe both the physical characteristics and the performance of the novel bait stations under semi-field and field conditions. In particular, we aimed at quantifying (1) the response of adult *B. dorsalis* to three different dilutions of GF-120 Fruit Fly Bait in order to identify the optimal concentration of GF-120 for use in association with these bait stations, and (2) the relative attractiveness of GF-120 aged for different time periods when in association with the novel bait stations to female *B. dorsalis*. The novel bait stations proven very effective in protecting GF-120 Fruit Fly Bait against rainfall, and therefore, they represent an alternative to foliar bait sprays under conditions of high rainfall. Compared to foliar sprays, the novel bait station enhances the behavioral response of fruit flies to GF-120 while increasing the longevity of GF-120. The bait station also reduces the amount of GF-120 used per hectare compared to foliar sprays, thereby providing an additional economic benefit to growers while reducing contamination of underground water. In conclusion, the novel bait stations have proven very effective in attract-and-kill of not only *B. dorsalis*, but also of *B. cucurbitae* (the melon fly). Therefore there is the potential of using this device against other economically important species of fruit flies. This technology represents for growers a simple, efficient and economical way of controlling fruit flies and a safer alternative to foliar sprays.

Managing Oriental Fruit Fly *Bactrocera dorsalis*, Using Spinosad-Based Protein Bait Sprays in Papaya Orchards in Hawaii

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Management of tephritid flies in many areas of the world has traditionally relied on frequent applications of broad spectrum insecticides. We evaluated the efficacy of sprays of GF-120 Fruit Fly Bait, in combination with two additional fruit fly management methods, in reducing populations of oriental fruit fly, *Bactrocera dorsalis*, in papaya orchards in Hawaii. Our main goal is to develop and implement environmentally acceptable and sustainable pest management strategies that reduce use of broad spectrum insecticides while suppressing fruit flies to economically manageable levels. The study was conducted in papaya orchards located in the Puna district of the island of Hawaii, in an area comprising 150 hectares in production. Fruit fly management tactics used included field sanitation (by growers), use of the highly attractive male-specific lure methyl eugenol and weekly sprays of GF-120 Fruit Fly Bait to papaya tree foliage and bordering plants. Results were assessed weekly using traps that monitored local populations of females and every 6 weeks by sampling fruit to determine level of infestation. Weekly applications of GF-120 Fruit Fly Bait, when combined with field sanitation and mass trapping of males, led to a significant reduction in both the number of females captured in monitoring traps and levels of fruit infestation of papaya fruit in the treated plots compared to the control plots. Parasitism by *Fopius arisanus* was not negatively affected by the applications of the protein bait. In Conclusion**:** An environmentally-friendly approach to managing Oriental fruit fly, *B. dorsalis* was evaluated with high success in an extensive area in Hawaii. With additional research currently being conducted, this technology will be transferred shortly to papaya growers in Hawaii.

Evaluation *in Vitro* of Potential Bio-Insecticidal Effects of Neem, Azadirachta *indica*, on Fruit Flies *Anastrepha obliqua* and *A. ludens*

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The fruit flies *Anastrepha obliqua* y *A. ludens* are economic important pests for the tropical fruits of the State of Veracruz, Mexico. These species survive autumn and winter season feeding on fruits of wild hosts that exist in small numbers in this region: fruits of these hosts commonly are consumed as fresh fruit immediately after harvested. Plantations of Neem have been increased in Veracruz, due to its popularity for human consumption and because of its insecticide properties. Therefore it was considered suitable to evaluate the effect of seeds-extracts of Neem in the mortality of adults of *A. ludens* and *A. obliqua* and also determining its effects on flies's fecundity, fertility and their success in reaching the adult state. Flies (sexually mature, both sexes) were exposed to concentrations of 3000, 4000 and 6000 ppm of alcoholic-watery extract, provided by oral route in the water to drink and by contact spreading it on the oviposición substrates during 10 consecutive days. The response variables were the mortality of individuals at the different stages: eggs, larva, pupal and adult (females and males). The concentration of 4000 ppm showed a partial effect in the studied variables, but the concentration of 6000 ppm showed a noticeable negative effect on the fecundity, fertility, and on the total efficiency in preventing that the adult state, after the first two days of exposition to the extracts. The effects on each species are discussed. The concentration of of seeds-extracts of Neem at 6000 ppm as potential treatment for combat of *A. obliqua* and *A. ludens* is considered suitable

Eficiencia del Tratamiento Hidrotérmico e Hidroenfriado y su Efecto sobre la Calidad del Mango Cv. Ataulfo de Chiapas Infestado con la Mosca del Mediterráneo, *Ceratitis capitata* (Diptera: Tephritidae)

Emilio Hernández, Bigail Bravo, Arseny Escobar, J. Pedro Rivera, José Manuel Gutiérrez, Pablo Montoya

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El tratamiento hidrotérmico por inmersión en agua caliente y enfriado al aire libre para mangos Cv. Ataulfo del estado de Chiapas infestados con *C. capitata* fue determinado con larvas de segundo y tercer estadio de una colonia de laboratorio establecida con moscas capturadas en Guatemala, que corresponden a poblaciones descritas por los haplotipos AAA, AAN y ANN. Existe evidencia de que el estadio más tolerante es la larva de primer instar. El objetivo de este trabajo fue validar el tratamiento comercial actual que consiste en sumergir los fruto en agua a temperatura inicial de 47° C, la cual al minuto 6 disminuye a 46.6 °C, al minuto 31 disminuye a 46.5°Cy en el minuto 50 disminuye a 46.5 permaneciendo hasta el final del tratamiento, con lo cual se logra que la temperatura de la pulpa del fruto al término del tratamiento alcance los 46.1° C e inmediatamente se enfría en agua a >23°C. Además se determinó su efecto sobre la calidad del fruto. Los resultados obtenidos con 30,000 individuos indicaron que el tratamiento hidrotérmico causa el 100% de mortalidad de huevos de 12 y 36 h, larvas de primer, segundo y tercer estadio en frutos infestados

en forma artificial. La mortalidad fue determinada con base en el número de individuos que terminaron su desarrollo hasta larvas de tercer estadio y que puparon en un periodo de cinco días después de su separación de la dieta. Los testigos de frutos infestados que no recibieron tratamiento hidrotérmico presentaron una sobrevivencia mayor del 90%, con lo cual se concluye que la mortalidad en los tratamientos fue causada por la inmersión en agua caliente. Los datos de calidad fueron determinados a los 3, 6, 9 y 12 días, indicaron que la inmersión de los mangos en agua caliente causó pérdida significativa de peso y firmeza. Se observaron cambios significativos en color, pH y grados Brix°.

Evaluación del Efecto Inhibidor del Quitosán en el Desarrollo de Diferentes Estados Inmaduros de la Mosca Mexicana de la Fruta *Anastrepha ludens.*

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El quitosan es un polímero que se obtiene por desacetilación parcial de la quitina. Se ha empleado como recubrimiento de semillas, como aditivo en alimentos para animales; como espesante en alimentos, entre otras cosas. Trabajos preliminares demostraron que el biorrecubrimiento de frutos de mango cv. Ataulfo con quitosan, además de inhibir el crecimiento de hongos fitopatógenos, inhibe el desarrollo de huevos de *Anastrepha ludens*. Para dilucidar si el quitosan directamente influye en el desarrollo de los huevos de *A. ludens*, o solo es un efecto de barrera, el objetivo del presente trabajo fue determinar el efecto del contacto directo del quitosan sobre el desarrollo de huevos y larvas de *A. ludens*. Se utilizó quitosan de alto, medio y bajo peso molecular, en concentración de 2, 1, 0.5 y 0.25%, disuelto en ácido ascórbico o acético o tartárico o láctico, al 0.1M. El huevo se expuso a las soluciones de quitosan en columnas de burbujeo. Las larvas se expusieron añadiendo la solución de quitosan a la dieta. Se encontró efecto biocida del quitosan sobre las larvas. Dicho efecto fue mayor en larvas de primer estadio, con quitosan de bajo peso molecular y con la mayor concentración del polímero. Por otro lado, en las larvas de primero y segundo estadio sobrevivientes se observó retraso en su desarrollo. En huevos no se observó modificaciones en la cinética de emergencia de larvas.

Cold Quarantine Treatment for Mediterranean Fruit Fly, *Ceratitis capitata* Control in Peppers

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SENASA (National Plant Protection Organization) in Argentina established methyl bromide fumigation as a desinfestation treatment for fruit flies in peppers (*Capsicum annuum*). Although this treatment is effective for *Ceratitis capitata* control, it affects the quality of the fruits, and has noxious effects on the environment. For this reason alternative quarantine treatments are looked for. The aim of this work was to develop a low temperature treatment for *C.capitata* control in peppers. The tests consisted on insect removal of fruits inoculated artificially with the most tolerant stage

(third instar larvae) to cold. Development of the quarantine treatment was carried out in two stages, small and large scale tests. The first set of tests included treatments of 3,000 viable insects at different exposure times (13, 14 and 15 days) at 1 ± 0,5 ºC. The second set of tests were done to confirm the elimination of more than 30,000 third instar larvae with the schedule achieved in the previous tests. After treating 40,336 third instar larvae at 1 ± 0,5 ºC for 14 days no viable larvae were found ($ED_{99.9926}$, 95 % confidence level). With this treatment at low temperatures the complete elimination of *C. capitata* was achieved and could be proposed as an alternative treatment to the fumigation with methyl bromide in pepper for its commercialization.

Sesión/Session IX

Control Biológico/Biological Control

Classical Biological Control Releases of the Natural Enemies, *Fopius arisanus* (Sonan) and *Diachasmimorpha longicaudata* (Ashmead), against Oriental Fruit Fly in French Polynesia

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Introduction

Oriental fruit fly (OFF), *Bactrocera dorsalis* (Hendel), is considered to be among the five most damaging and aggressive pest fruit flies in the world. (Leblanc and Putoa 2000). It is distributed throughout Asia, including Bhutan, southern China, India, and Thailand (White and Elson-Harris 1992). *Bactrocera dorsalis* is native to tropical Asia and has been accidentally introduced into the Commonwealth of the Northern Mariana Islands in 1935, Hawaii in 1945, Guam in 1948, Nauru in the 1980's, and Tahiti in 1996 (Leblanc and Putoa 2000). One hundred twenty-four hosts of *B. dorsalis* have been recorded for tropical Asia (Allwood et al. 1999). Recently, two species in the *B. dorsalis* complex have become established on two new continents: *Bactrocera carambolae* Drew and Hancock, the carambola fruit fly, in Suriname in South America, and *Bactrocera invadens* Drew, Tsuruta & White in Kenya in Africa (Drew et al. 2005; Rousse et al. 2005).

Four economically important fruit flies have become accidentally established in French Polynesia: *Bactrocera kirki* (Froggatt) in 1928, *B. tryoni* (Froggatt), Queensland fruit fly, in 1970, *B. dorsalis*, in 1996, and *B. xanthodes*, Pacific fruit fly, in 1998 (Leblanc and Putoa 2000). At the time of these studies, *B. dorsalis* had been reported only in the Society Islands and *B. xanthodes* only in the Austral Islands. Circumstantial evidence suggests that *B. dorsalis* was introduced into French Polynesia from Hawaii. Large-scale eradication programs were conducted on Tahiti and Moorea Islands in 1997. They included applications of methyl eugenol (Steiner et al. 1965) and protein bait (Steiner 1952) with insecticides. After many treatment campaigns, *B. dorsalis* populations were reduced to a few small pockets on both islands. Nonetheless, by 2001, *B. dorsalis* populations rebounded and, additionally, spread to other French Polynesia islands of the Society Island group including Raiatea, Tahaa, and Huahine. In 2007 it was recovered in the Marquesas Islands. Reported here are studies on the impact of the *B. dorsalis* natural enemies, *Fopius arisanus* (Sonan) and *Diachasmimorpha longicaudata* (Ashmead), introduced into French Polynesia from Hawaii.

Materials and Methods

Fruit Fly Abundance and Their Host Fruits

Fruits of *Psidium guajava* L. (guava), *Inocarpus fagifer* (Parkinson) Fosberg (Polynesian chestnut), *Terminalia catappa* L. (tropical almond), and *Mangifera indica* L. (mango) trees were commonly encountered and collected along major roadways of Tahiti Island. Other host fruits in various quantities were collected sporadically throughout the year, but due to unpredictable fruiting patterns and scattered nature of trees along roadsides, numbers of fruits sampled varied. Fruits were weighed and placed in batches on wire metal screen (43 by 28 by 6 cm) inside plastic holding boxes (50 by 32 by 15 cm) that contained 1.5 cm of fine sawdust and were held for 3 wk. Sawdust from fruit holding boxes was sifted weekly. Pupae were transferred to smaller plastic containers and held until emergence of flies or parasitoids. Fruits and recovered pupae were held in a room maintained at 22 ± 5°C, ambient (40-90%) RH, and a 12:12 (L:D) h photoperiod. Numbers of fruit flies and parasitoids that emerged were recorded.

Impact of *F. arisanus* Releases

Fopius arisanus wasps were from a colony maintained for 150 generations at the United States Department of Agricultures (USDA), Agricultural Research Service (ARS), Pacific Basin Agricultural Research Center (PBARC) facility in Honolulu, HI. Ten shipments of *F. arisanus* (of approximately 50,000 each for a total of 523,127 wasps) inside fruit fly pupae were sent by airplane to Tahiti lsland between December 2002 and October 2004. Similarly, five shipments of *D. longicaudata* (of approximately 5,000 each for a total of 10,000 wasps) were sent between April 2007 and August 2008. Parasitiods were transferred from Faaa International Airport to the Service du Développement Rural Laboratory in Papara, Tahiti. A small laboratory was established at Papara for evaluating parasitism in the field, rearing small numbers of fruit flies, and rearing wasps for augmentative releases of *F. arisanus* according to the methodology of Harris et al. (2000).

Data Processing and Statistical Methods

For *P. guajava*, *I. fagifer*, and *T. catappa* fruit collections throughout Tahiti Island, data for numbers of *B. dorsalis*, *B. tryoni*, and *B. kirki* recovered from fruits were pooled by year (1998 to 2006), and fruit fly species per kg calculated. Percent parasitism by *F. arisanus* (number of adult *F. arisanus*/number of adult *B. dorsalis* + *B. tryoni* + *B. kirki* + *F. arisanus*) was calculated for the years 2003 to 2006. To determine the impact of *F. arisanus* on fruit fly species, fruit fly (*B. dorsalis*, *B. tryoni*, and *B. kirki*) emergence per kg of fruit was compared for 2002 (before *F. arisanus* releases) and 2006 (after *F. arisanus* releases) as a percent decrease in infestation for *P. guajava*, *I. fagifer*, and *T. catappa* fruit collections. Limited preliminary data were evaluated for *D. longicaudata.*

Results

Parasitism and Suppression of Fruit Flies.

Fopius arisanus was recovered from 15 different host plant species. For collections of *P. guajava* fruits for Tahiti Island, *F. arisanus* parasitism was 2.1, 31.8, 37.5, and 51.9% for fruits collected during 2003, 2004, 2005, and 2006, respectively (Table 1). From 2002 (before parasitoid releases) to 2006 (after parasitoid releases), there was a subsequent decline in numbers of fruit flies emerging (per kg of fruit) by *B. dorsalis*, *B. tryoni*, and *B. kirki* of 75.6, 79.3, and 97.9%, respectively. For all collections of *I. fagifer* fruits for Tahiti Island, *F. arisanus* parasitism of fruit flies was 2.4, 9.2, 38.8, and 42.0% for 2003, 2004, 2005, and 2006, respectively. From 2002 to 2006 there was a subsequent decline in numbers of fruit flies emerging (per kg of fruit) by *B. tryoni* and *B. kirki* of 69.0 and 94.0%, respectively. There was no decline in numbers of *B. dorsalis* emerging from this fruit. For all collections of *T. catappa* fruits for Tahiti Island, *F. arisanus* parasitism of fruit flies was 0.6, 5.6, 12.3, and 49.8% for 2003, 2004, 2005, and 2006, respectively. From 2002 to 2006 there was a subsequent decline in numbers of fruit flies emerging (per kg of fruit) by *B. dorsalis*, *B. tryoni*, and *B. kirki* of 65.8, 80.2, and 91.8 %, respectively. During 2006, mean (± sd) *F. arisanus* parasitism for fruit flies infesting *P. guajava*, *I. fagifer*, and *T. catappa* fruits was 47.9 ± 5.2 %. Although *F. arisanus* was released along the coast in only nine communities, it spread rapidly and was recovered from 21 of 21 communities on Tahiti Island in 3 yr. Similarly, after releases on other islands, *F. arisanus* was reared from fruits collected on Moorea, Huahine, Tahaa, and Raiatea Islands.

On Tahiti Island, to increase fruit fly parasitism rates, a second species of parasitoid, *D. longicaudata*, a larval-pupal parasitoid, was introduced into French Polynesia in April 2007. In March 2008 *D. longicaudata* was confirmed established in Tahiti. Thus far, *D. longicaudata* has been recovered from breadfruit, *Artocarpus altilis* (Parkinson) Fosberg *P. guajava*, *I. fagifer*, and *T. catappa* fruits (Table 2).

Discussion

Parasitism of Fruit Flies

In surveys of Kauai Island in Hawaii, the egg-pupal parasitoid, *F. arisanus*, constituted 87.5-95.1% of the parasitoid guild and was very common in tree fruits, particularly *P. guajava* and *P. cattleianum* (Vargas et al. 1983b, 1993). Consequently, this species was selected first for introduction into French Polynesia. On Tahiti Island, *F. arisanus* became established throughout the island in 21 of 21 communities within 3 yr. On the basis of *P. guajava*, *I. fagifer*, and *T. catappa* fruit collections, parasitism has averaged approximately 50%. In addition, *F. arisanus* became quickly established on the other Society Islands of Moorea, Raiatea, Tahaa, and Huahine by shipping small cages of parasitoids bred from field collected infested fruit, and releasing them in *C. papaya* orchards or in wild *P. guajava* patches.

Perhaps no fruit fly parasitoid has been as successful in suppressing host populations as *F. arisanus* (Rousse et al. 2005). Because of its habit of attacking host eggs, which are more exposed to parasitism than larvae, it can achieve high levels of parasitism, often surpassing 50% in the field (Vargas et al. 1993; Purcell et al. 1998). The success of classical biological control against fruit flies in Hawaii, in particular with *F. arisanus*, has been thoroughly reviewed by Rousse et al. (2005). In Hawaii, the impact of *F. arisanus* introductions resulted in a 95% reduction in the *B. dorsalis* population, compared to the 1947-1949 peak abundance of *B. dorsalis* (DeBach and Rosen 1991). Furthermore, *F. arisanus* became the major parasitoid of *C. capitata* in Hawaii (DeBach and Rosen 1991; Vargas et al. 2001). Haramoto and Bess (1970) reported that the mean number of fruit fly pupae (*B. dorsalis* and *C. capitata*) collected from coffee, *Coffea arabica* L., fruits in Kona, HI decreased from 23.6 pupae per 100 fruits (8.7% parasitism) in 1949 to 5.2 (66.6% parasitism) in 1969. With this level of impact on infestation level, establishment of *F. arisanus* has reduced the threat of movement of fruit flies to the mainland from Hawaii.

In the present study we were able to compare fruit samples before and after releases of *F. arisanus* on Tahiti Island. From 2002 (before parasitoid releases) to 2006 (after parasitoid releases), there has been a decline in numbers of fruit flies emerging (per kg of fruit) by *B. dorsalis, B. tryoni*, and *B. kirki* of 75.6, 79.3, and 97.9%, respectively. We recognize that much of the decline in numbers of *B. tryoni* and *B. kirki* may have been due to competitive interactions with *B. dorsalis*. However, *F. arisanus* may have also played a role in the decline. The impact of *F. arisanus* releases has not always been as impressive in other locations outside of Hawaii to date (Rousse et al. 2005). For example, *F. arisanus* has been released and recovered in Costa Rica, but the impact has not been high, although little information is available on its present status or distribution on *C. arabica* farms with *C. capitata* infests fruits (Wharton et al. 1981). Similarly, in Australia, *F. arisanus* was introduced from Hawaii and was established on the native *B. tryoni* in 1962, but reputedly had only a negligible effect (Quimio and Walter 2001). Its introduction to control native pest fruit flies on various islands in Fiji and western Polynesia has resulted in generally less than 10% parasitism (Allwood 1997). Nonetheless, establishment of *F. arisanus* in French Polynesia against *B. dorsalis* is now the most successful example of classical biological control of fruit flies in the Pacific area outside of the Hawaiian Islands and serves as a model for introduction of the parasitoid into South America and Africa, where *B. carambolae* and *B. invadens* (Drew et al. 2005) have recently become established. In addition, *F. arisanus* is being studied as a possible candidate for classical biological control of the peach fruit fly, *Bactrocera zonata* (Saunders), in Africa and the Indian Ocean region (e.g. FAO/IAEA 2005).

French Polynesia is comprised of over 118 islands and atolls scattered over approximately 2,500,000 km^2 of ocean. Until 2007, when *B. dorsalis* spread to the Marquesas Islands, *B. dorsalis* was confined to the Society Islands. Initially it was envisioned that *F. arisanus* could be mass reared at an estimated cost of US $2,000 per 1,000,000 parasitoids (Harris and Bautista 2001), and transferred to other islands as *B. dorsalis* spread throughout French Polynesia. However, when *F. arisanus* became numerous

in fruits infested with *B. dorsalis* on Tahiti Island, it became more cost effective to recover wasps from fruits held inside screened cages, and ship them to the outer islands, than to mass rear them in the laboratory on artificial diet. This approach is now being used for shipments to the Marquesas Islands where *B. dorsalis* has recently spread. Nonetheless, for approximately $100,000 US, the shipment and establishment of *F. arisanus* in French Polynesia has provided a sustainable program to reduce the impact of *B. dorsalis* that was not achieved with expensive eradication programs. Consequently, establishment of *F. arisanus* has reduced the threat of movement of fruit flies to the mainland from French Polynesia.

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Footnotes

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Table 1. *P. guajava* fruit collection data for Tahiti Island from 1998 to 2006 showing number of collections, number of fruits collected, fruit weight, total pupae recovered, number of pupae per kg, species recovered, species per kg and % *F. arisanus* parasitism (after Vargas et al. 2007).

Year	No. collec-tions	No. fruit	Weight (kg)	Total No. Pupae	No. pupae/kg fruit	No. *B. dorsalis*	No. *B. dorsalis* /kg fruit	No. *B. tryoni*	No. *B. tryoni* /kg fruit	No. *B. kirki*	No. *B. kirki* /kg fruit	No. *F. arisanus*	Parasitism (%)
1998	15	176	2.5	17,150	7,014.3	23	9.4	9,939	4,065.0	1,063	434.8		
1999	30	304	26.9	1,022	38.1	0	0.0	464	17.3	220	8.2		
2000	20	203	15.9	1,097	69.1	0	0.0	1,025	64.6	72	4.5		
2001	7	70	7.9	1,300	164.4	151	19.1	842	106.5	117	14.8		
2002	60	641	48.1	11,306	234.9	4,868	101.1	2,049	42.6	687	14.3		
2003	165	1,678	130.0	47,783	367.6	21,940	168.8	8,340	64.2	894	6.9	677	2.1
2004	230	2,344	171.0	53,084	310.4	15,150	88.6	6,126	35.8	420	2.5	10,111	31.8
2005	103	1,074	65.0	15,917	244.8	3,558	54.7	2,123	32.7	124	1.9	3,479	37.5
2006	145	1,484	136.2	16,801	123.4	3,360	24.7	1,202	8.8	43	0.3	4,971	51.9

Table 2. Fruit collection data for Tahiti Island from January to May 2008 showing host, number of collections, number of fruits collected, fruit weight, total pupae recovered, number of pupae per kg, species recovered, and % *F. arisanus* (*F. a.*) and % *D. longicaudata* (*D. l.*) parasitism.

Host	No. collec-tions	No. fruit	Weight (kg)	No. pupae	No. *B. dorsalis*	No. *B. tryoni*	No. *B.kirkii*	No. *F. a.*	No. *D. l.*	% emergence	% *F. a.*	% *D. l.*
Artocarpus altilis (Parkinson) Fosberg (bread fruit)	6	27	59.0	498	27	0	0	39	6	14.5	54.2	8.3
Averrhoa carambola L (star fruit)	4	77	5.0	10	6	0	0	1	0	70.0	14.3	0.0
Carica papaya L (papaya)	9	16	14.2	312	51	0	0	66	0	37.5	56.4	0.0
Citrus sp	5	20	9.6	34	25	0	0	3	0	82.4	10.7	0.0
Inocarpus fagifer (Parkinson) Fosberg (Polynesian chestnut)	43	1,118	104.7	11,798	3,569	290	3	2,772	62	56.8	41.4	0.9
Mangifera indica L (mango)	41	800	186.1	3,233	876	23	0	570	0	45.4	38.8	0.0
Musa sp (banana)	20	158	11.3	1,315	625	3	0	197	0	62.7	23.9	0.0
Passiflora edulis Sims (passion fruit)	6	28	1.9	260	119	0	0	43	0	62.3	26.5	0.0
Persea americana Mill. (avocado)	7	25	9.5	67	11	10	0	6	0	40.3	22.2	0.0
Pometia pinnata J. R. Forster & G. Forster (Pacific lychee)	4	39	3.2	792	490	10	0	81	0	73.4	13.9	0.0
Psidium guajava L (common guava)	73	2,615	243.0	17,677	2,222	545	21	7,031	3	55.6	71.6	0.0
Syzgium cumini (L) Skeels (Jambolan plum)	1	59	0.4	5	0	2	0	0	0	40.0	0.0	0.0
Terminalia catappa L (tropical almond)	31	3,270	81.7	10,777	411	1,389	99	3,641	7	51.5	65.6	0.1

Resúmenes de Posters Presentados/Abstracts of Presented Posters

Control de *Ceratitis capitata* en Zonas Cafetaleras Mediante Liberaciones de Adultos Estériles Transmisores de Conidios de *Beauveria bassiana* (Bals.) Vuill

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Dentro de las actividades de contención que realiza el Programa Moscamed en la franja fronteriza México-Guatemala, y como una alternativa a la oposición social que existe en esta zona a las aspersiones aéreas de insecticida para el control de dicha plaga, se evaluó la actividad biológica del hongo entomopatógeno *Beauveria bassiana* sobre brotes de *Ceratitis capitata* en zonas cafetaleras de Chiapas, liberando adultos estériles tratados con conidios del hongo como vectores. Se seleccionaron siete aéreas con presencia de plaga, de las cuales una fue tomada como testigo y en el resto se realizó la liberaron de adultos estériles inoculados (800 o mas adultos/Ha) en una proporción de 10 g de conidios por kg de mosca (~200,000 adultos/kg). Los adultos tratados para ser liberados se colocaron en bolsas de papel no. 20 en una proporción de 5,000 adultos voladores por bolsa. La liberación se efectuó vía aérea y/o terrestre de la semana 16 a la 42 de 2007. Los adultos silvestres capturados con trampas Jakson y Fase IV fueron llevados al laboratorio, donde se cuantificaron y se colocaron en cámaras húmedas para promover el desarrollo del hongo. De las 50 moscas silvestres capturadas, el 75% de las hembras y el 64% de los machos presentaron esporulación de *B. bassiana*. En los adultos capturados en la zona testigo no hubo presencia de micosis. Lo anterior es una evidencia de que la liberación de machos de *C. capitata* (capa TSL) inoculados con *B. bassiana*, es una estrategia potencial que complementa el manejo integrado de esta plaga. Esta técnica puede tener su mayor utilidad en agroecosistemas zonas de agricultura orgánicos como es el caso de las zonas productoras de café, en donde *B. bassiana* está permitido su uso y puede coadyuvar en el control de poblaciones de la broca del café.

Efecto del "Cake" de Nim (*Azadirachta Indica*) en la Mosca de la Fruta *Ceratitis capitata* (Diptera: Tephritidae) y su Parasitoide *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae)

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Fue evaluada la supervivencia y el índice de parasistismo de *Diachasmimorpha longicaudata* a partir de larvas de *Ceratitis capitata* previamente parasitadas y posteriormente expuestas a la acción

del nim. Para tal, las larvas del 3º instar de *C. capitata* fueron envueltas en tejidos tipo voil ("unidades de parasitismo") y expuestas al parasitismo en el interior de una jaula conteniendo entre 400 a 600 pares de parasitoides sexualmente maduros (con 5 días de edad). Las larvas fueron expuestas al parasitismo de *D. longicaudata* por un periodo de una hora y posteriormente fueron acondicionadas en vasos descartables con vermiculita, conteniendo diferentes cantidades (proporciones) del cake de nim (0%, 5%, 10%, 15%, 20%, 25% e 30%). Para evaluar la emergencia de *C. capitata*, las larvas del mismo lote utilizado fueron inoculadas en los recipientes-tratamiento, sin parasitismo previo. El diseño estadístico fue completamente aleatorizado, con cuatro repeticiones, siendo cada parcela constituida por 100 larvas. Fueron evaluados el número de adultos emergentes (moscas o parásitos) y el índice de parasitismo. La emergencia de parasitoides y el índice de parasitismo fueron afectados negativamente con el aumento de la proporción de nim, lo que fue observado para todos los tratamientos que recibieron el cake de nim. La emergencia de moscas provenientes de larvas no parasitadas y expuestas a diferentes proporciones de cake de nim se redujo significativamente en la medida que hubo un aumento de la proporción del insecticida botánico. Cuando las larvas fueron previamente parasitadas y expuestas al nim, la emergencia de *C. capitata* fue reducida en 56,5%, en relación a lo observado para el tratamiento con la mayor proporción de nim. El nim posee acción de contacto al parasitoide afectando el índice de parasitismo, sin embargo, su uso asociado al control biológico proporciona una mayor reducción en la emergencia de *C. capitata*.

Entomopathogenic Fungi Used on *Anastrepha obliqua* Young Adult-Fruit Flies (Macquart) (Diptera: Tephritideae)

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Biological control of fruit flies is an important tool for pest management in fields with high populations. Enthomopatogenic fungi could be an easy and efficient technique in biological control. Fifteen isolates from entomopathogenic *Beauveria bassiana* (Balsamo) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok. fungi were evaluated in the Tolima's University Entomology and Phytopathology laboratories on one day-old adult *Anastrepha obliqua* (Macquart) for selecting the most pathogenic strains; this was aimed at contributing towards developing biological control techniques for this pest. The isolates were obtained from LAVERLAM SA and CENICAFE's laboratories and commercial products. A screening with a $1x10^7$ conidia/ml concentration was used for selecting the three most pathogenic isolates, two from *Beauveria* (TR10 and TR8) and one from *Metarhizium* (TR3), having 77%, 71% and 66% mortality, respectively. No significant differences were found between females and males in terms of mortality. The LC_{50} for each isolates was $2.38x10^6$, $1.81x10^6$ and $9.94x10^6$ conidia/ml respectively and the respective LT_{50} was 48.12, 56 and 42.75 hours. The LC_{90} of selected isolates was spraying on fly pupation medium led to 5%-48% mortality being obtained during the 120 hours of evaluation. Our results shown that there is a commercial fungal biopesticide available to use in *Anastrepha obliqua* pest management in Colombia and it could be used aim to young adult.

Especies de Parasitoides (Hymenoptera: Braconidae) Asociados a *Anastrepha* spp. (Diptera: Tephritidae) en Frutos Hospederos de la Región Litoral Sur del Estado de Bahia.

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Este trabajo tuvo por objetivo verificar las especies de Braconidae asociados a las frutas cultivadas en las provincias de la región Litoral Sur de Bahia: Camamu (13º 58' S; 39º 0,8' W; 26m), Ituberá (13º 47' S; 39º 12' W; 110 a 140m), Taperoá (13º 33' S; 39º 12' W; 160m) y Valença (13º 20' S; 39º 10' W y 130 a 190 metros). En el período de agosto de 2005 a marzo de 2008, se han colectados frutos de acerola (*Malpighia punicifolia* – Malpighiaceae), abiu (*Pouteria caimito* – Sapotaceae), carambola (*Averrhoa carambola* – Oxalidaceae); de la familia Myrtaceae: guayaba (*Psidium guajava*) y el pitanguero (*Eugenia uniflora*); y de la familia Anacardiaceae los frutos de cajá (*Spondias mombin*), cajarana (*S. cytherea*), serigüela (*S. purpurea*) y mango (*Mangifera indica*). Los frutos colectados fueron colocados sobre una cama de vermiculita, en bandejas plásticas. Semanalmente, los puparios fueron separados del substrato, retirados y acondicionados en frascos plásticos conteniendo vermiculita, siendo cubiertos con *voile* y mantenidos en cámara climática (BOD) con temperatura de 25 ± 1ºC, permaneciendo en observación hasta la eclosión de los dípteros (moscas de las frutas) y, o de parasitoides. Fueron obtenidos los siguientes especímenes del género *Anastrepha*: *A. fraterculus*, *A. obliqua*, *A. bahiensis*, *A serpentina*, *A. sororcula* e *A. zenildae*. Del total de 838 ejemplares de braconidos, 19,11% fueron de los especímenes *Utetes anastrephae* (Viereck), provenientes del cajá, carambola, guayaba, mango y pitanga; 4,27% de la espécimen *Asobara anastrephae* (Muesebeck) obtenidos de los frutos del cajá, carambola y guayaba, y solo un ejemplar de la espécimen *Opius* aff. *bellus* (0,20%) que emergió de la muestra de guayaba. El espécimen *Doryctobracon areolatus* (Szépligeti) (76,42%) fue el predominante y emergió de los puparios provenientes de todos los frutos hospederos colectados, probablemente por la mayor eficiencia de esta especie en localizar las larvas de los tefrítidos.

Infección de *Anastrepha ludens* con el Hongo *Beauveria bassiana* Aplicado en Suelo con Diferentes Rangos de Humedad y Temperatura

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Se evaluó el efecto de un producto a base de conidios de *Beauveria bassiana* sobre la emergencia de *A. ludens* en suelos areno-arcillosos con diferentes rangos de humedad y temperatura. Cada unidad experimental contó con 100 pupas próximas a emerger, registrando la mortalidad de los adultos durante 10 días consecutivos. Los experimentos se realizaron a nivel de laboratorio con 27 ± 2 ºC, 80 ± 5% H.R., y 12:12 horas luz: oscuridad con 6 repeticiones. Para corroborar la infección

del hongo las moscas muertas se colocaron en cámara húmeda. En el primer experimento se aplicaron 0, 0.4, 0.6, 0.8, 1.2 y 1.6 g de conidios (diluidos en 40 mL de agua)/kg de suelo con 12% de HR, del cual se colocó una capa de 0.5 cm en recipientes de plástico de 14.5 x 22.5 cm donde se colocaron las pupas; posteriormente se colocó el remanente del suelo hasta formar una capa de 4.5 cm de profundidad. De los diferentes tratamientos se obtuvo un rango de infección de 38.3 a 74.7%. En otro experimento se aplicó 0.8 g de conidios/kg de suelo con 6, 9, 12, 15, 18, y 21% de humedad. La infección obtenida varió de 43.0 a 79.8%, correspondiendo al suelo con 12% de humedad la mayor infección y con 21% la menor mortalidad. Finalmente se aplicó 0.8 g de conidios/kg de suelo con 12% de humedad, a 15, 20 25, 30 y 35 ºC. La infección de adultos varió de 82.5 hasta 91.0%; la mayor actividad biológica fue a 25 ºC, aunque las diferencias entre tratamientos no fueron significativas. Aplicando 0.8 g de conidios/kg de suelo de textura areno-arcillosa con 12% de humedad y a 25 ºC, se registró la mayor infección de adultos de *A. ludens*.

Rearing, Life History, and Seasonal Abundance Studies on *Opius bellus* (Hymenoptera: Braconidae, Opiinae), an *Anastrepha* spp. Parasitoid

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Opius bellus is a widespread Neotropical, koinobiont, larval endoparasitoid of *Anastrepha* genera. Only data on distribution, taxonomy, host fly range, host fruit association, and abundance in the field were previously published for this fruit fly parasitoid. Due to the importance of *O. bellus* as a parasitoid of *Anastrepha fraterculus* in NW Argentina, we have much interest in establishing for first time a laboratory rearing of this wasp in the insectary of PROIMI. Therefore, we made a systematic fruit sampling throughout four years (September/1999 to August/2003) in a secondary forest area belong to Yungas' subtropical rainforest, located in Tucumán. We collected five host fruit species: *Juglans australis* (Juglandaceae), *Citrus aurantium* (Rutaceae), *Psidium guajava* (Myrtaceae), *Eriobotrya japonica*, and *Prunus persica* (Rosaceae). Several specimens of *O. bellus* were obtained from *A. fraterculus* pupae collected from *P. guajava* and *P. persica*, and from mixed pupae of *A. fraterculus* and *A. shultzi* collected from *J. australis*. *Opius bellus* was present in the field from December to April, and it had a diapause period from May to November. *Opius bellus* was successfully developed on artificially reared *A. fraterculus* larvae in the Laboratory. Three different oviposition substrate types were gradually utilized to colonize this parasitoid species: 1) fruit guava filled with host larvae, 2) artificial oviposition units filled with host larvae plus fruit guava pulp, and 3) artificial oviposition units only filled with host larvae. The *O. bellus* adults used in the life history studies stemmed from a colony that was 14 generations old. Main daily fecundity was (2.04 $\pm$ 0.04) female offspring by parental female for maternal age intervals from 19 to 21 days. Sex ratio was (226.90 $\pm$ 279.20) for the progeny of F_{14}, and development time of immature stages at 25± 1ºC was (25.75$\pm$ 2.76) for females, and (24.33 $\pm$ 1.51) for males.

Functional Response Analysis of *Diachasmimorpha longicaudata* (Ashmead) Reared on *Anastrepha fraterculus* (Wiedemann)

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A bioassay with the parasitoid *Diachasmimorpha longicaudata* reared on third instar *Anastrepha fraterculus* larvae at 25 ± 1 ºC, 75 ± 5% RH, and 12:12 (L:D) h photoperiod was carry out in order to determine the functional response parameters. The study was performed in the Insectary of PROIMI-CONICET, Biological Control Division, San Miguel de Tucumán, Argentina. Eight *A. fraterculus* larvae densities were tested independently (1, 2, 3, 5, 15, 30, 60 and 120 larvae per Petri dish without larval diet and covered with mesh). Each larval density was exposed during 3 hours to a single 6-8 days old randomly selected parasitoid female. The densities 1 and 2 were replicated 71 times, densities 3 and 5 were replicated 70 and 68 times respectively, and densities 15 to 120 larvae were replicated 41 times. Low densities were replicated more times in order to obtain a better discrimination between a type II and III functional response curve. Before the experiment, the parasitoids were held in 13x20x25 cm plastic frame cage at a density of ~100 parasitoid, 1:1 sex rate per cage, and they were provided with water and honey. A density-dependence in the proportion of larvae attacked was found, so we fitted the data to the equation proposed by Hassell et al. (1977). The following parameters were recorded: 1) percentage of total parasitism (emerged plus unmerged parasitoids) at different densities; 2) functional response parameters; handling time (Th) and searching rate (a'). A type III functional response was found. Functional response measuring in laboratory may show little resemblance to those obtained on field, but is important to take account, that it could be used to infer basic mechanisms underlying the parasitoid-host interaction, so this kind of experiments could provide important information for biological control programs.

Natural Parasitism in Fruit Flies in Livramento de Nossa Senhora, Bahia, Brazil.

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The Livramento de Nossa Senhora fruit growing area, in the southwest of State of Bahia has presently 11,000 hectares of mangos and fruit flies are the major pest species affecting the production. Natural parasitoids of fruit flies are important factors as biological control agents and there was no information about the species that occurs in the region and their relation with the fruit host. Fruits of 30 plant species were collected between June 2006 and December 2007 according their availability in the field and brought to laboratory for fruit fly pupa recover and parasitoids emergence. From 14,721 pupa reared from 23,440 fruits, 861 parasitoids were obtained. From the 30 fruit species, only 17 had fly infestation and from those, five has shown parasitoid in the pupa. Red mombin (*Spondias lutea*) had 33.8% of infestation, followed by cajarana (*Spondias spp*) 8.1%, yellow mombin (*S. purpurea)* 4.5%, umbu (*S. tuberosa*) 4.0% and jua (*Zizyphus joazeiro)* 1%. The

parasitoids emerged from the pupa were *Dorytobraon aerolatus* (91,1%), *Utetes anastrephae* (6.5%), *Asobara anastrephae* (2.4%).

Colonization and Domestication of Seven Species of Larval-Prepupal and Pupal Native New World Hymenopterous Fruit Fly (Diptera: Tephritidae) Parasitoids

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Techniques used to colonize and domesticate seven native New World species of hymenopterous parasitoids that attack flies within the genus *Anastrepha* (Diptera: Tephritidae) were developed: *Doryctobracon areolatus* (Szépligeti), *Doryctobracon crawfordi* (Viereck), *Opius hirtus* (Fischer), *Utetes anastrephae* (Viereck) (all Braconidae, Opiinae), *Aganaspis pelleranoi* (Bréthes) and *Odontosema anastrephae* Borgmeier (both Figitidae, Eucoilinae) (all larval-pupal parasitoids), and the pupal parasitoid *Coptera haywardi* (Ogloblin) (Diapriidae, Diapriinae) were reared successfully on Mexican fruit fly, *Anastrepha ludens* (Loew) larvae or pupae reared on artificial diet. Descriptions of the different rearing techniques used throughout the domestication process are described to help researchers to colonize local parasitoids. We also describe handling procedures such as number of hosts in parasitization units and compare optimal host and female age, differences in parasitism rate, developmental time, life expectancy and variation in sex ratios in each parasitoid species over various generations. In the case of *D. crawfordi* and *C. haywardi* we also provide partial information on mass-rearing techniques such as cage type, parasitization unit, larval irradiation dose and adult handling.

Sesión/Session X

Procedimientos Regulatorios y Enfoque de Sistemas/Regulatory Procedures and Systems Approach

Sistema de Mitigación de Riesgo en Areas de Escasa Prevalencia para la Movilización de Frutos Hospederos de *Ceratitis capitata* (Wied.) con Destino a Areas Libres de la Provincia de Mendoza

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Introducción

En la República Argentina de las especies de moscas de los frutos de importancia cuarentenaria, únicamente se ha reportado la presencia de *Anastrepha fraterculus* (Wied.) y *Ceratitis capitata* (Wied).

En la provincia de Mendoza sólo se ha detectado la "Mosca del Mediterráneo" *Ceratitis capitata* (Wied.) y el PROCEM-MENDOZA desarrolla el Programa de Control y Erradicación para esta plaga. Cuenta con un Sistema de Detección permanente para esta especie y otras moscas de la fruta desde el año 1991, un sistema de protección cuarentenaria y un plan de acciones de control sustentado en la aplicación de la Técnica del Insecto Estéril (TIE), complementado con acciones de control químico y cultural.

Mendoza posee un clima continental árido-desértico, con bajo nivel pluviométrico (90 a 350 mm anuales) y sus cuatro oasis productivos cuentan con importantes barreras naturales, la Cordillera de los Andes al Oeste y rodeados por extensas áreas desérticas con típica vegetación xerófila, sin hospederos naturales para las moscas de los frutos. La superficie cultivada es de 272.850 has. con vid, frutales, hortalizas y otros, correspondiéndole el 50% a la vid y el 25% a frutales.

Avances

El PROCEM-MENDOZA ha obtenido los reconocimientos del Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA) de:

- "Área de Escasa Prevalencia de Moscas de los Frutos" a la Provincia de Mendoza (Disposición SENASA Nº 01/02)
- "Área Libre de Mosca del Mediterráneo" a los Valles de Malargüe y El Sosneado en San Rafael (Disposición SENASA Nº 06/03)
- "Área Libre de Mosca del Mediterráneo" al Valle de Uco - Oasis Centro (Disposición SENASA Nº 15/04)
- "Área Libre de Mosca Sudamericana de la Fruta" a la Provincia de Mendoza (Disposición SENASA Nº 05/05)
- "Área Libre de Mosca del Mediterráneo" a San Rafael y General Alvear – Oasis Sur (Disposición SENASA Nº 07/06)

Además, el reconocimiento de "Área Libre de Mosca del Mediterráneo" de los Valles de Malargüe y El Sosneado en San Rafael y del Valle de Uco - Oasis Centro, por el Servicio Agrícola y Ganadero de Chile (Resolución SAG Nº 5331).

Esta situación hizo que la Provincia quedara dividida en dos grandes áreas, una con estatus de Área de Escasa Prevalencia (AEP), Oasis Norte y Este y otra con estatus de Área Libre de Mosca de los Frutos (ALMF), Oasis Centro y Sur. Consecuentemente surgieron inconvenientes comerciales al restringir el ingreso de hospederos de *Ceratitis capitata* (Wied.) de los oasis Norte y Este a los oasis Centro y Sur de la Provincia, tanto para consumo en fresco como para industrialización.

La superficie cultivada de frutales en los oasis Norte y Este es de 24.150 has. con una producción estimada de 257.800 ton. Parte de esta producción se industrializa en establecimientos ubicados en el ALMF constituyendo una importante actividad económica de la provincia de Mendoza como es la agroindustria y parte se comercializa para consumo en fresco.

Por ello se diseñó un procedimiento que permitiera el ingreso de hospederos de *Ceratitis capitata* (Wied.) desde las Áreas de Escasa Prevalencia (AEP), que garantizara la protección de las Áreas Libres de Mosca de los Frutos (ALMF) mitigando el riesgo de introducción de la plaga.

Se estableció un Sistema de Mitigación de Riesgo (SMR) para frutas producidas en AEP con destino a consumo en fresco en ALMF y otro para fruta producida en AEP con destino a industrialización en ALMF, con medidas integradas para el manejo del riego de la plaga.

SMR para fruta con destino consumo en fresco en ALMF

Los procedimientos establecidos son los siguientes:

- Inscripción en el sistema del productor interesado en movilizar la producción.
- Trampeo y muestreo del cultivo inscripto, como mínimo 35 días antes de cosecha.
- Si desde la fecha de inicio del monitoreo y antes de inicio de la cosecha no se detectan adultos en trampa, ni larvas en frutos, se autoriza la cosecha, se colocan en las cajas cosecheras las tarjetas de identificación (productor, nº de inscripción, especie, variedad y fecha), se verifica la carga con resguardo y documentación del transporte que traslada la partida al establecimiento de Empaque. El procedimiento se realiza en presencia del inspector del PROCEM.
- El establecimiento de empaque para las partidas autorizadas, previamente habilitado, debe tener un responsable técnico quien hará el muestreo de las partidas ingresadas y registro de ingreso/egreso de las mismas, tener las áreas de recepción y almacenamiento debidamente aisladas, disponer de un área de inspección de lotes y todo el proceso de empaque se hace en presencia de un inspector del PROCEM, quien identifica cada uno de los envases terminados con un sello. Los empaques cuando trabajan con fruta del SMR, no pueden trabajar fruta de otra procedencia.
- Cuando los envases son despachados a las ALMF, el inspector del PROCEM verifica la carga, el resguardo del transporte, emite el Certificado de Partida Libre y precinta el transporte.
- Antes del ingreso a las ALMF, es verificada la documentación y el resguardo de la partida en los Puestos de Control Interno, donde se archiva el Certificado de Partida Libre y se sella el remito. Se realiza un muestreo de la partida y se coloca un nuevo precinto dejando aclarado el número del mismo en el certificado correspondiente y se autoriza en ingreso al ALMF.
- El ingreso de fruta del AEP para consumo en fresco en las ALMF, tiene una fecha límite, considerando la mínima probabilidad de presencia de la plaga en el AEP de la provincia de Mendoza, en función del monitoreo histórico.

SMR para fruta destino a industrialización

Los procedimientos establecidos son los siguientes:

- Inscripción en el sistema del productor interesado en movilizar la producción.
- Trampeo y muestreo del cultivo inscripto como mínimo 35 días antes de cosecha.
- Si desde la fecha de inicio del monitoreo y antes de inicio de cosecha no se detectan adultos en trampa, ni larvas en frutos, se autoriza la cosecha, se colocan en las cajas cosecheras las tarjetas de identificación (productor, nº de inscripción, especie, variedad y fecha) y se verifica la carga con resguardo y documentación del transporte que traslada la partida a la industria ubicada en el ALMF.

- Antes del ingreso de las partidas al ALMF, en los Puestos de Control Interno, se verifica la documentación, se realiza un muestreo, se coloca un precinto y se emite el Certificado de Partida Libre.
- En el establecimiento industrial, un inspector del PROCEM verifica la documentación, retira el precinto y autoriza el inmediato proceso industrial.

Si durante el desarrollo de los sistemas descriptos, se detectara en el monitoreo adultos en trampas o estados inmaduros en fruta o estados inmaduros en los muestreos de fruta en los empaques o en los muestreos de fruta en los Puestos de Control Internos, se da de baja del sistema al establecimiento agrícola y darle otro destino a la producción, fuera del área protegida o realizar tratamiento cuarentenario.

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Fruit Flies Risk Analysis, Current Situation and Perspectives

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Trade in fresh agricultural commodities involves probability of entry and establishment of exotic organisms into the importing region or country. The term "risk" includes the product of likelihood that exotic organisms will enter and become established (survive and reproduce) in the importing region and the costs of this introduction (environmental or economic). In agricultural trade, risk is usually expressed as the product of likelihood of introduction and establishment as a probability and the cost in money of dealing with the introduction. The cost may be costs of management of a new pest, financial cost of eradication, or environmental costs due to ecological changes the introduction entails.

Pest Risk Analysis is recognized as the primary step in evaluating the need for quarantine actions for these imports. The procedures for evaluating pest risk in USDA APHIS is reviewed in Devorshak and Griffin and was discussed in the context of the systems approach for consideration of host status in Aluja and Mangan (2008). Devorshak and Griffin (2002) reviewed agreements under the International Plant Protection Convention (IPPC). These documents describe the standards for controlling spread and introduction of plant pests are found in a series of International Standards for Phytosanitary Measures(ISPM) given in (IPPC 2004). In particular ISPM 11 (2005) gives guidelines for the pest risk analysis process and ISPM 14 (2002) gives guidelines for the systems approach. Although these documents give guidelines for evaluating, developing and presenting the analysis for quarantine actions, the actual scientific, environmental and economic considerations as well as the actual operation of the system is dependant on the conditions in the exporting and importing regions and the biology of the pests and the hosts.

The development of alternatives to single treatments to achieve quarantine security was largely due to the withdrawal of ethylene dibromide as a fumigant for human health reasons (Anon. 1984). This was followed by proposed restrictions for use of methyl bromide as a fumigant for environmental reasons (Anon. 1993). The combination of loss of two important fumigants used for quarantine treatments and the increased interest in alternatives being proposed by trade agreements such as NAFTA combined to raise quarantine treatment research to a high level of urgency for USDA. Two of the earliest publications that addressed host resistance and non-host status as alternatives to fumigations (Greany et al. 1983, Landolt et al. 1983) showed that grapefruit and other citrus are poor hosts for *Anastrepha suspensa* and these studies were the basis for the management zones used in place of fumigation in Florida (Riherd et al. 1994).

A series of meetings were held in 1992 and 1993 to assess all options for alternatives to these fumigants. After a series of documents were produced such as Annon (1993b) containing biological, economic and industry summaries, a collection of reviews were edited and published by two USDA ARS scientists (Sharp and Hallman 1994). The term "Systems Approach" was introduced as one chapter (Jang and Moffitt 1994) as well as chapters discussing various quarantine treatments including statistical methods (Chew 1994), heat and cold treatments (Gould 1994), irradiation (Burditt 1994), fumigation (Yokoyama 1994), hot water (Sharp 1994) and combination treatments (Mangan and Sharp 1994). Other chapters address ecological and pre-harvest conditions that can be components of the systems approach such as including, effects of climate on exotic pest establishment (Worner 1994), pest free areas (Riherd et al. 1994) and commodity resistance to infestation (Armstrong 1994). This publication led a series of studies reviewing literature and addressing approaches to replace the bromide fumigants.

A recent publication (Sgrillo 2005) defines a number of the concepts or factors and presents quantitative approaches to development and discussion of phytosanitary regulations. The Sgrillo publication, coupled with the ISPMs 11 and 14 and the practical discussion by Sequeira (2002) of agricultural risk assessment can give researchers and regulatory officials an overview of factors involved in risk analysis and the systems approach. Among the most apparent themes of these discussions is that from a regulatory point of view, general definitions and procedures for are desirable for developing regulations (Follett and Neven 2006), but in practice the relations of pests, host commodities and production conditions must be considered on a species by species and habitat by habitat basis and the general approaches to defining systems approaches are limited in use (Sequiera 2002).

Research approaches to host status, host status relative to fruit maturation, possible genetic variation within host and pest species, and the ecological factors that affect maintenance of pest populations and commodity infestation are needed for development of systems approaches for many commodities. Information concerning the biology of host use by pests is particularly needed in cases involving proposals for export under a systems approach. As discussed by Sgrillo (2005) importing countries have the sovereign right to choose the acceptable level of risk (ALR) for pest introduction in imported commodities. Although the importer has this right, the biological factors needed the importer to develop an ALR are not likely to be available from biologists from the importing country since the pest is usually not present in the country or at least in the area needing quarantine protection. Under these conditions, the exporting region must supply reliable data showing how the proposed exported commodities will not pose a risk to the importer.

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Resúmenes de Posters Presentados/Abstracts of Presented Posters

The Use of Irradiation as a Phytosanitary Measure for Fruit Fly Exclusion: Program Requirements, Facility Certification, and Treatment Verification Methods

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In the United States, the use of irradiation for phytosanitary purposes has long been contemplated as a viable alternative to conventional quarantine treatments. However, acceptance and the practical application of this technology has been limited given that the objective of this treatment is the prevention of the establishment of pests whereby pest mortality is not necessary to achieve this goal. This presents unique challenges when it comes to verifying treatment efficacy at ports of entry, and once irradiated commodities have entered US commerce. The regulation developed by APHIS in 2002 and revised in 2006, provides the framework for importing irradiated articles from foreign countries. It describes a means by which APHIS certifies foreign irradiation facilities to ensure minimum target dose can be accurately delivered and verified once the commodity has been treated. The regulation also describes safeguards to protect the product against potential infestation prior to entry into the United States. Irradiation Programs are transformed into operational practice through Preclearance programs. Components of the Preclearance Operational Work Plan include methods to minimize the risk of infestation via field mitigation strategies, preclearance inspection regimes, treatment monitoring procedures, pre and post-treatment safeguarding provisions, and product labeling and trace back requirements. Irradiation treatment facilities are certified by APHIS after review of standard operating procedures, safeguards, record keeping, and verification of dosimetry systems. APHIS maintains an online system to track irradiation treatments and facilitate traceback of specific irradiated consignments shipped to the US. The aforementioned regulations and program parameters developed by APHIS provide a high level of phytosanitary security while facilitating safe trade in irradiated articles and ensuring system integrity as described in the International Standard (ISPM 18).

Patagonia Argentina, Área Libre de Moscas de los Frutos

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La Patagonia Argentina es una vasta región con una superficie de 816.291 km^2 y cerca de 1.700.00 habitantes. Existen 150.000 has. de valles irrigados que conforman verdaderos oasis dentro de la planicie semiárida, característica de la región patagónica. De ellas, más de 70.000 se encuentran en plena producción fruti-hortícola, principalmente frutas de pepita, carozo y frutas finas, con excelentes condiciones de calidad para su exportación. La región produce cerca de 1.000.000 de toneladas de

manzanas, 600.000 de peras, y 75.000 de carozo. Todo el área se encuentra aislada geográficamente por extensas superficies de montes xerófito achaparrado. El río Colorado conforma su límite norte, a lo largo de más de 1.000 km, donde 13 Puestos de Control actúan como una prevención adicional al ingreso de productos de origen animal o vegetal de carácter restringido o prohibido. En puertos marítimos y aeropuertos, se controla el ingreso de cargas y pasajeros a la región. El Sistema de Detección del Programa semanalmente revisa más de 2.400 trampas y toma más de 700 muestras semanales de fruta, en plena temporada de trabajo, trabajan en el Programa, más de 85 personas, entre profesionales y técnicos de campo y laboratorio. El Programa presenta un Programa Preventivo de liberación de mosca estéril complementado con un Plan de Emergencia Fitosanitaria que contempla Acciones Localizadas de Control Químico y Cultural ante la ocurrencia de una detección, y de Regulación Cuarentenaria. La Región Patagónica fue reconocida como Área Libre de Mosca de los Frutos por el USDA-APHIS en diciembre de 2005, lo que permitió al sector exportador ahorrar cerca de 2 millones de dólares anuales en tratamientos cuarentenarios, además de diversificar su oferta incluyendo la cereza entre los productos exportados, y el libre ingreso por cualquier puerto a los Estados Unidos.

Sistema Cuarentenario Patagónico

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El Sistema Cuarentenario Patagónico es ejecutado y administrado por la Fundación Barrera Zoofitosanitaria Patagónica, entidad no gubernamental compuesta por el SENASA, los Gobiernos provinciales de la región y las Asociaciones y Federaciones de Productores Agropecuarios. Su objetivo es evitar el ingreso de plagas y enfermedades perjudiciales para la producción agropecuaria de la región protegida, aplicando las medidas y acciones zoofitosanitarias necesarias para establecer, proteger y mantener el área libre de plagas y enfermedades con reconocimiento internacional, contribuyendo además con la fiscalización de la calidad y sanidad agroalimentaria. La legislación que se aplica en el Sistema Cuarentenario Patagónico es de índole nacional emanada del Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA) y de la Secretaria de Agricultura, Ganadería, Pesca y Alimentos de la Nación (SAGPyA). El Sistema está compuesto por la barrera de los ríos Colorado y Barrancas, la barrera del río Negro, Confluencia, la barrera del Paralelo 42º, Ferrocarriles, Aeropuertos y Puertos de la Patagonia. Tres patrullas volantes se suman a los cuarenta y cinco puntos de control que componen el Sistema. Está representado por trescientas personas entre Profesionales, Técnicos e Inspectores que son capacitados previo al ingreso y luego evaluados anualmente. La labor de los inspectores está complementada por el Programa Incan Trehua (perros protectores) perteneciente a la Funbapa. compuesto por canes entrenados para la detección de productos orgánicos. El financiamiento del Sistema Cuarentenario Patagónico está dado por los fondos originados a partir del arancel por control, inspección y desinsectación que se cobra en los puestos de barrera terrestres, aportes del Gobierno Nacional y las Provincias.

Aplicación del Concepto Area Libre de Plagas para Moscas de la Fruta en México

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Uno de los objetivos de la Campaña Nacional contra Moscas de la Fruta es reconocer áreas libres de *Anastrepha ludens, A. obliqua, A. striata y A. serpentina*, a efecto de exportar frutas sin tratamiento cuarentenario de postcosecha. En este sentido y con base en lo previsto en la Ley Federal de Sanidad Vegetal, NOM-023-FITO-1995, NOM-075-FITO-1997, NIMF no. 4 y NIMF No. 26, de 1992 a 2008 el Gobierno de México ha reconocido 920,570 km^2 como áreas libres de moscas de la fruta (ALP-MF). Australia, Estados Unidos, Nueva Zelanda, Unión Europea y Japón han reconocido 202,017 km^2 como ALP-MF al Estado de Baja California Sur, 19 municipios de Sonora, seis municipios de Chihuahua y cinco municipios de Sinaloa. Actualmente, se continúan las gestiones para ampliar el reconocimiento internacional de ALP-MF. Para el mantenimiento de las ALP-MF, anualmente, se tienen en operación 7,000 trampas con revisión semanal y 35 Puntos de Verificación Interna donde se han retenido y destruido 600 ton de frutas; se han muestreado 500 ton de frutas; se fumigaron 120 mil ton de frutas; y se inspeccionaron 10.5 millones de vehículos. Asimismo, se realiza inspección fitosanitaria en 19 aeropuertos, tres puertos y principales mercados. Ante la detección de la plaga, se implementa el plan de emergencia hasta lograr su erradicación. Adicionalmente, se aplica control de calidad del trampeo mediante la colocación controlada de moscas marcadas en las trampas. Durante 2007, bajo el concepto de ALP-MF, se exportaron 27 mil ton de mango, 19 mil ton de naranja y 1,200 ton de durazno, con un valor comercial de 17, 6.7 y 3 millones de dólares, respectivamente. El costo anual del manejo de las ALP-MF es de 3.5 millones de dólares; de los cuales, el 52% se invierte en Regulación Cuarentenaria, 40% en Vigilancia Fitosanitaria y 8% en el Plan de Emergencia.

RSPM No. 27. Guidelines for Importation and Confined Release of Transgenic Arthropods in NAPPO Member Countries

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The technology for development of transgenic arthropods is well established and transgenic arthropod strains are available in laboratories for evaluation and potential future use in some plant pest control programs conducted in North American Plant Protection Organization (NAPPO) member countries. NAPPO Regional Standard for Phytosanitary Measures (RSPM) No. 27 was adopted in October 2007 to provide guidance on the importation and confined field release of transgenic arthropods that are known plant pests or have the potential to affect plant health. Regulatory decisions regarding transgenic arthropods should be science-based and made on a case-by-case basis. Authorizations may require that:

- The applicant provides sufficient information to identify the transgenic arthropod, the type of action proposed, and the proposed and available risk management options.
- The National Plant Protection Organization (NPPO) conducts a pest risk analysis (PRA) of the phytosanitary risk potential associated with the unmodified recipient arthropod and the transgenic arthropod.
- The NPPO determines that specific PRA criteria and any risk management options intended to minimize potential phytosanitary risk associated with the transgenic arthropod have been met.

RSPM No. 30. Guidelines for Determination and Designation of Host Status of a Fruit or Vegetable for Fruit Flies (Diptera: Tephritidae)

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The objective of host status trials is to demonstrate host status of a specified fruit or vegetable based on statistically valid data. Although protocols and comprehensive trials to determine fruit fly host status have been documented in the scientific literature, inconsistencies in terminology and methodologies have contributed to discrepancies in assessing pest risk and in application of phytosanitary measures. NAPPO Regional Standard for Phytosanitary Measures No. 30 harmonizes terminology and protocols for determining fruit fly host status in order to promote consistency among NAPPO member countries and supporting scientific communities. Host status designations are:

- Natural host – A fruit or vegetable that becomes infested by a plant pest in nature (e.g., natural, cultivated and/or unmanaged plants) and the plant pest population is sustained on the fruit or vegetable. No other trials are necessary to confirm host status.
- Natural non-host – A fruit or vegetable that does not become infested by a plant pest in nature (e.g., natural, cultivated and/or unmanaged plants) and the plant pest population is not sustained on the fruit or vegetable.
- Conditional host – A fruit or vegetable that is host or a non-host under defined permissive or restrictive conditions, respectively (e.g., stage of maturity, other physiological conditions, physical conditions).

RSPM No. 30 provides guidelines for conducting laboratory, field cage, glasshouse, and natural field infestation trials. Field and laboratory experiments should be representative of variability in the fruit or vegetable and fruit fly populations over the entire growing, harvest, and export period and area. Experiments should be replicated, statistically analyzed, and the levels of confidence reported based on sample size so that data is verifiable and replicable. Specification of the defined condition(s) of the fruit or vegetable to be evaluated as a resistance factor(s) is essential to the designation of conditional host status.

Programa de Control y Erradicación de Mosca de los Frutos (Procem) - Argentina

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La Republica Argentina se caracteriza por la preponderancia de las economías regionales. El sector frutihortícola uno de los rubros más importantes, con el 20% del PBI agrícola del país. La producción se destina tanto al mercado interno como al de exportación. Este último genera divisas por 1000 millones de dólares. Debido a la importancia de este sector, en el año 1994, se puso en marcha el Programa Nacional de Control y Erradicación de Mosca de los Frutos. El PROCEM ejecuta acciones de control para *Anastrepha fraterculus* y *Ceratitis capitata* y de exclusión para especies de importancia cuarentenaria tales como: *Ceratitis* spp., *Anastrepha* spp., *Toxotrypana curvicauda*, *Bactrocera* spp., *Rhagoletis* spp. *y Dacus* spp.

Cultivo	Superficie en hectáreas
Carozo	67.124
Pepita	59.828
Cítricos (sin limón)	96.965
Uva	192.488

Actividades:

- **Red de detección permanente de alta sensibilidad**
- **Acciones de Control**: TIE, control químico y cultural.
- **Sistema de Protección Cuarentenaria**: 24 horas -todo el año- se realizan tareas de control e inspección (con detectores de productos orgánicos y/o perros adiestrados). Además se realiza muestreo cuarentenario de especies frutihortícolas que ingresan a las Áreas Bajo Programa
- **Centros de Tratamiento Cuarentenario:** se localizan fuera de las Áreas Bajo Programa, y efectúan los tratamientos cuarentenarios aprobados a los frutos hospederos con destino a estas áreas.
- **Producción de Mosca Esteril**: existen dos bioplantas (Provincias de Mendoza y San Juan) las cuales proveen el material a las Áreas Bajo Programa.

Logros:

Área Libre de Mosca de los Frutos*:* Oasis Centro y Sur de la Provincia de Mendoza y Región Patagónica.

Área Libre de *A. fraterculus* y de Escasa Prevalencia de *C. capitata*: Oasis Norte y Este de la Provincia de Mendoza.

Área de Escasa Prevalencia de *C. capitata* y Bajo Control de *A. fraterculus*: Valle de Calingasta (Provincia de San Juan).

Área Bajo Control de *C. capitata* y *A. fraterculus*: Valles productivos de San Juan y La Rioja.

Áreas de Diagnostico*:* Macizo Citrícola Monte Caseros (Provincia de Corrientes)-Colon (Provincia de Entre Ríos) y Valle del Conlara y Llanura Norte (Provincia de San Luis)**.**

Personal Afectado: 1100 entre profesionales y técnicos. Hectáreas Bajo Programa: 1.273.600 has.

Desarrollo de un Proceso Industrial Basado en Altas Presiones Hidrostáticas para Eliminar la Mosca de la Fruta en Mango de Exportación

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La alta presión hidrostática (APH) inactiva microorganismos patógenos y deterioradores de alimentos ocasionando daño mínimo al producto, retención de frescura, sabor, textura y color. Su efecto es uniforme e instantáneo a través del alimento, por lo tanto independiente de la geometría y tamaño lo que permite el fácil escalamiento y una rápida transferencia tecnológica de los resultados de laboratorio a procesos industriales. La APH ha sido estudiada para eliminar larvas de insectos o nemátodos en alimentos: para

eliminar al parásito *Anisakis simplex*, nematodo presente en peces; larvas de *Trichinella spirallis*, nemátodo presente en carne de cerdo; huevecillos y larva de la mosca de la fruta del mediterráneo (*Ceratitis capitata*), polilla de la manzana *Cydia pomonella* y mosca de la fruta de la cereza *Rhagoletis indifferens*. No existen estudios del empleo APH para destruir los huevecillos de larvas de la mosca de la fruta mexicana (*Anastrepha ludens*), sin alterar la fruta de interés, que en el presente estudio es mango. Estudios reportados en la literatura establecen que los huevecillos de primer día y las larvas en tercer estadío son más resistentes a las APH, requiriéndose condiciones de 125 MPa por 10 a 20 minutos a temperaturas cercanas a 25 °C para su eliminación. El objetivo de este proyecto es establecer la viabilidad y rentabilidad de aplicar las altas presiones hidrostáticas en el tratamiento del mango poscosecha, para la eliminación de la mosca de la fruta, como alternativa a los procesos hidrotérmicos. Los resultados preliminares muestran que los mangos tipo bola en estado maduro se deterioraran notablemente a 100 MPa y se modifican ligeramente a 75 MPa. Los mangos parecen soportar condiciones inferiores a 50 MPa, en el rango de 0 a 50 °C por tiempos de hasta 20 min.

Technical Considerations of Systems Approaches for Trade of Fruit Fly Hosts Products

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Tephritid fruit flies are considered quarantine pests by regulatory agencies of many countries and thus commodities that are considered fruit fly hosts in international trade must meet the regulatory requirements of the importing country. In most cases, results of Pest Risk Assessments (PRAs) identify fruit flies as a high risk thus generating the need for quarantine treatments or other mitigating procedures. Systems approaches encompass the full range of biologically-based management and treatment alternatives that could result in the step-wise reduction of risk starting with planting and production through field management, harvest, packing, shipping and distribution at destination to sequentially mitigate risk of introduction. A number of technical considerations must be considered in the development of systems approaches to alleviate this risk. Each system approach will likely be different depending on the crop, pests, incidence of the pest in the crop during various periods of the growing through harvest cycle and influence of adjacent hosts to the planting areas. This may require an area-wide management strategy as part of the systems approach. Quarantine treatments may also play an important role where the risks of introduction are particularly high and/or need for security approaches 100%. In this case treatments with efficacy of less than probit 9 (99.9968%) may work as part of the systems approach. While systems approaches will likely require more attention to detail than individual quarantine treatments, they nonetheless represent a biological way to manage risk of fruit fly introduction.

Aplicación de Medidas Integradas en un Sistema de Mitigación de Riesgo (SMR) para *Anastrepha grandis* (McQuart) en la Producción de Cucurbitáceas con fines de exportación, desde el Dpto. de Concepción, Paraguay a la República de Argentina

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El propósito de este trabajo fue cumplir con los lineamientos y las regulaciones fitosanitarias establecidas por la Rca. Argentina para la importación de frutos frescos de Sandia (*Citrullus lanatus*) y Calabacita (*Cucúrbita moschata*) procedentes del Dpto. de Concepción, Rca. del Paraguay, mediante la aplicación de un Sistema de Mitigacion de Riesgo para *Anastrepha grandis* (Mosca Sudamericana de las Cucurbitáceas) en los distritos de Concepción, Horqueta, Loreto y Belén del citado Departamento. Este trabajo fue realizado en sucesivos periodos agricolas (2006-2007-2008), participando 241 productores con una superficie total de 208 hectáreas inscriptas en el programa de exportaciones. En los tres periodos de producción se realizaron monitoreos (trampeo y muestreo), utilizándose 325 trampas tipo Mc Phail y pellets de proteína hidrolizada tipo "torula" como atrayente alimenticio. Fueron muestreados 789,88 Kg. de frutas. La frecuencia de recebado de las trampas fue de 5 días (2006/07) y 7 días (2008). En el Dpto. de Concepción fue montado un Laboratorio de Identificación de Moscas de la Fruta donde fueron analizadas e identificadas especies del genero *Anastrepha* y las muestras fueron procesadas a fin de detectar estados inmaduros de la plaga. Los resultados obtenidos señalan que no fueron detectados en trampas ni estados inmaduros de *Anastrepha grandis* en los periodos evaluados. Además se han capturado e identificado cinco especies del genero *Anastrepha: A. fraterculus* (60,97 %), *A. montei* (19,51 %), *A. Pseudoparalella* (14,63 %), *A. barbiellini* (2,44 %) y *A. dissimilis* (2,44 %). La no detección de estados inmaduros en frutas o captura de adultos en trampas se relacionaría con las condiciones ambientales desfavorables a la especie en los lugares estudiados. De las informaciones generadas, fue elaborado el MTD (mosca trampa día) para *Anastrepha grandis* y todas las especies identificadas. También fueron habilitadas plantas de empaque para el procesamiento y selección de frutas con destino a la exportación.

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