The South American fruit fly, Anastrepha fraterculus (Wied.); advances in artificial rearing, taxonomic status and biological studies

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FOREWORD

Many fruit fly species are identified as worldwide pests of great concern to the fruit production and export industries. In the Americas, they have been and remain the cause of huge monetary investments, not only for control by fruit growers but also for exclusion and/or eradication efforts through government programmes.

From an economic and social point of view, fruit production and export industries are very important for many countries in the hemisphere, mainly because these activities are important sources of employment and foreign exchange. However, in addition to direct damage to fruit in the field, the presence of pest fruit flies has an important indirect negative impact on the development of these industries.

One of the fruit flies of major concern, because of its economic and quarantine importance in the Americas, is the exotic Mediterranean fruit fly, *Ceratitis capitata*, which is established throughout the Central and South American countries, excluding Chile. Chile, Mexico and the USA have conducted multi-million dollar campaigns to prevent the establishment of this and other exotic fruit flies in their respective territories, in support of the development of important fruit production and export industries. Other important fruit fly species, which are native to the American continent, are those of the genus *Anastrepha*. In this group, of most economic importance are *A. obliqua* and *A. ludens* for Mexico and some Central American countries and *A. fraterculus* and *A. obliqua* for South America.

In this publication, attention is focused on *A. fraterculus*, the South American fruit fly. This species, as it is presently recognized, occurs from Mexico to Argentina and is reported from approximately 80 host plants, including commercial fruits of economic importance, such as mango, citrus, guava, apple and coffee.

In the large fruit production areas of Uruguay, Argentina and Peru in which *A. fraterculus* and *C. capitata* are the only fruit fly species of economic and quarantine importance reported, the development of technologies and strategies for control and/or eradication of both species is of great interest. The sterile insect technique (SIT), to control and/or eradicate *C. capitata*, has been developed and successfully applied in Argentina, Chile, Peru, Mexico and the USA. If no effective control methods are developed against *A. fraterculus*, this could, in the near future, greatly reduce the benefits of *C. capitata* control or eradication in those areas where the two species are presently found.

As *A. fraterculus* is considered to be of high economic and quarantine importance in many countries in South America, it is justifiable to recommend and promote the implementation of activities to strengthen knowledge of the species and develop techniques for its control and/or eradication. The development of SIT and other biological control methods are very encouraging alternatives, as can be seen from examples in Mexico and the USA, where these approaches are in use against *A. ludens* and *A. obliqua*.

Many studies have been conducted on the biology, behaviour, genetics and taxonomy of *A. fraterculus* in South America. There have also been serious attempts to mass rear the fly. There are working papers containing knowledge of predators and parasitoids of the species. However, all of these studies, even those that represent very important contributions,

have been isolated and often lack continuity. Efforts have been duplicated, and the limited resources assigned to this issue have not been rationally utilized. As a consequence, there have been only minor advances in methods development to effectively control the pest — depending still on traditional control methods with traps and toxic baits.

The integration of efforts amongst countries, through a co-ordinated strategy to study and develop effective control methods against this pest, is a task of the highest priority. The fruit production activity of affected countries is of high value and should be protected with environmentally friendly control methods. The continuous use of insecticides to control these pests should be reduced, and SIT and biological methods should be developed as alternatives. IT has long demonstrated its great effectiveness against *C. capitata* and more recently against *Anastrepha* species in Mexico.

The creation of *fruit fly free zones*, wherever technically and economically feasible or the creation of *fruit fly low prevalence zones*, would result in important benefits to many countries, given their existing potential to produce and export fruit of high quality to international markets, an outlet presently restricted due to the presence of these fruit flies.

In view of the above, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, which has long been involved in the development of SIT and complementary methods to control and/or eradicate *C. capitata* in many parts of the world, organized, with the support of the Agriculture Service (SAG) of Chile, a workshop. The objectives of this workshop were to assemble scientists and technicians from across the Americas who have had experience in relation to *A. fraterculus* and other *Anastrepha* species, in order to exchange information, analyse and discuss the present status of studies on this pest, and promote the integration and co-ordination of efforts to improve pest control techniques.

It is hoped that this publication will contribute to the integration of the technical and scientific efforts of the participating countries, which is needed to accelerate the development of more effective, environmentally responsible methods and strategies of pest control or eradication.

The IAEA officer responsible for this publication was G. Ortiz of the Joint FAO/IAEA Division.

EDITORIAL NOTE

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SUMMARY

The Workshop on the South American Fruit Fly, *Anastrepha fraterculus* (Wiedemann), held in Viña del Mar, Chile, in November 1996, was the first effort in Latin America to assemble fruit fly scientists and pest control programme managers from different countries in order that they could contribute with their experience and knowledge and at the same time acquire valuable information on the biology, behaviour, taxonomy and control methods of this important agricultural pest.

The event was organized by the IAEA in conjunction with the Government of Chile and attended by 18 fruit fly specialists invited by the Insect and Pest Control Section of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, in addition to 47 scientists and technicians from the continent participating as observers. This large number of participants reflected the importance of the event and the interest to improve co-ordination in research issues as well as in the knowledge of the control methods.

It was confirmed during the workshop that *Anastrepha fraterculus* is one of the most significant fruit fly pests in the Neotropical region and represents a threat to continuing agricultural development if management strategies are not identified and implemented.

An important debate took place on the taxonomic status of this fruit fly as various sources indicate that the populations of the species known as *A. fraterculus* actually includes more than one cryptic species. This variation not only creates problems for quarantine control measures of plant protection, but also makes the development of control and eradication methods especially difficult. This is particularly true for those methods based on the sterile insect technique (SIT), which rely on the correct selection of the target species to ensure that the production and release of sterile males coincide with the particular species present in the target area. Fundamental to the success of any effort made in an area-wide control programme will be the resolution of this taxonomic problem. To date, there is general agreement that *Anastrepha fraterculus* populations are not one species, but a complex of cryptic, or morphologically very similar species. To clarify the number of fruit fly species that exist within what is now called *Anastrepha fraterculus*, morphological as well as genetic analysis should be made with a central co-ordination and the collaboration of interested countries.

The specialists in artificial rearing of fruit flies were convinced that *Anastrepha fraterculus* has very positive perspectives to be a candidate for mass rearing and use in SIT programmes, mainly because it is a polyphagous species with characteristics similar to *Ceratitis capitata*. They also stated, that given the different regions of the continent occupied by the species, and the possibility of the existence of different subspecies, colonization and artificial rearing of *A. fraterculus* should be considered independently for each region, depending on the economic and quarantine importance related to this species.

Ongoing efforts in artificial rearing of *A. fraterculus* were reported by participants of Argentina, Brazil, Colombia and Peru, in which the low quantity of egg production and larvae diets were the main coincidental problems discussed. There was a very interesting exchange of information among the specialists from Mexico and Texas, USA, with those from the South American countries with regard to the positive experiences in mass rearing programmes of *Anastrepha ludens* (the Mexican fruit fly) and *Anastrepha obliqua* (the mango and plum fruit fly), especially in those aspects related to oviposition cages and base formulations for *Anastrepha spp.* larvae. They established proper channels of communication for continued interaction in these matters and for scientific visits to the institutes and laboratories in which artificial rearing of these fruit flies is in progress.

Interesting information was provided on the geographic distribution of the species with a review of several bibliographic sites. The species is reported from Southern Texas in the USA to Argentina and Chile. Chile gave a presentation on the eradication of the pest from their country in 1964. It was reported also that *A. fraterculus* is one of the most important fruit pests in Latin America, and, as currently defined, it is one of the most widely distributed and polyphagous species of the genus. With regard to biology and behaviour, important contributions were made from Brazil, Colombia and Argentina. Reported was the importance of this fruit fly which attacks apples in Brazil, a wide diversity of fruit species in Argentina and in Colombia. It is defined as the most important fruit fly, the coffee berry being its preferable host, but also mangoes and guavas. There was still some confusing information on host range and preferences. While in some countries *A. fraterculus* is reported attacking grapefruits, in others this fruit host is not attacked. This type of information has been reported repeatedly and it was therefore recognized as a priority in the near future to identify range, phenology and host associations of *A. fraterculus*, as well as obtaining samples for the morphological and genetic analysis required in order to provide the baseline data by which taxa can be identified. Most of the participants were interested in collaborating in this activity.

In order to make good comparisons among biotypes of *Anastrepha fraterculus* from different regions, the convenience for establishing the proper protocols to standardize laboratory and field studies, e.g. the use of specific type traps for fruit fly population studies, etc., was discussed. Fruit sampling surveys and studies to identify the level of preference of the fly for a particular host fruit were recommended as preliminary field studies that would greatly assist in the design of improved methods of control.

Lastly, studies on the reproductive compatibility of the different biotypes or fly populations of different origins, either with fertile specimens in countries without risk of infestation, or using sterile specimens, were discussed and recommended.

The group recommended the development of bio-rational control options, including the sterile insect technique (SIT) and biological control methods against this fruit fly species and the re-evaluation of quarantine restrictions for *A. fraterculus*.

TAXONOMY, GENETICS AND MOLECULAR BIOLOGY

LA MOSCA SUDAMERICANA DE LAS FRUTAS, *Anastrepha fraterculus* (WIEDEMANN) EN EL BRASIL

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Abstract–Resúmen

THE SOUTH AMERICAN FRUIT FLY, *Anastrepha fraterculus* (WIEDEMANN) IN BRAZIL.
    Anastrepha fraterculus, the South American fruit fly, is the most common and economically important pest for the fruit-bearing species in the Neotropical region. However, there are some species that are close to *A. fraterculus* and, sometimes they can be erroneously identified as *A. fraterculus*. The separation of *A. fraterculus* from *A. obliqua, A. sororcula* and *A. zenildae*, species closely related to South American fruit fly, is discussed. Also, information on the host plants and braconid parasitoids for *A. fraterculus* in Brazil is presented.

LA MOSCA SUDAMERICANA DE LAS FRUTAS, *Anastrepha fraterculus* (WIEDEMANN) EN EL BRASIL.
    Anastrepha fraterculus, la mosca Sud-Americana de las frutas, es la plaga mas comun y economicamente importante en la produccion de especies de frutas de la region Neotropical. Sin embargo, existen algunas especies muy cercanas a *A. fraterculus* y, algunas veces pueden ser erroneamente identificadas como *A. fraterculus*. La separacion de *A. fraterculus* de *A. obliqua, A. sororcula* y *A. zenildae,* especies estrechamente relacionadas a la mosca Sud-Americana de las frutas, es dicutida. Tambien, se presenta informacion sobre las plantas hospederas y los parasitoides braconidos para *A. fraterculus* en Brasil.

1. INTRODUCCION

    Tradicionalmente, en la literatura agrícola brasileña, *Anastrepha fraterculus* ha sido considerada la espécie más común en el país. Entretanto, hasta la década del 70, la denominación de las espécies de *Anastrepha* era bastante confusa y, apesar del trabajo de Lima [1], los estudios eran conducidos sin acompañamiento taxonómico. En la década del 60 *A. obliqua* (como *A. mombinpraeoptans*) era considerada como la espécie más común e importante en el Brasil. Posteriormente, casi hasta el final de la década del 70, *A. fraterculus* y *Ceratitis capitata* pasaron a ser consideradas las espécies más comunes y economicamente importantes en este país. En realidad, el uso del nombre específico era aquel disponible en la literatura, una vez que, en los trabajos aplicados, no existía la preocupación de confirmar la identidad de las espécies vinculadas a los mismos y tampoco habia la costumbre de mantener *voucher specimens*. Es debido a esto que nunca fue posible confirmar la identidad de las espécies referidas en vários trabajos, entre los cuales uno clásico como el de Puzzi & Orlando [2]. De esa forma, las informaciones incluidas en esos trabajos quedaron seriamente comprometidas por no ser posible conocer, con seguridad, las espécies realmente estudiadas.

Al final de la década del 70, la importáncia de la taxonomía de *Anastrepha* comenzó a ser más valorisada. A partir del trabajo de Zucchi [3], los levantamientos fueron basados en la identificación taxonómica y, ademas, las publicaciones y diversos entrenamientos propiciaron mayor conocimiento de la taxonomía de *Anastrepha* entre los entomólogos brasileños.

Diversos trabajos han mostrado que *A. fraterculus* es realmente una espécie común y ampliamente distribuída en el Brasil, mas en determinadas épocas y regiones otras espécies del grupo *fraterculus* pueden ser más abundantes que la propia mosca suramericana y por tanto, tambien importantes economicamente.

Este trabajo tiene por objetivo discutir al taxonomía clásica de *A. fraterculus*, sus plantas hospederas y sus braconídeos parasitóides en el Brasil.

2. TAXONOMIA

La mosca suramericana de las frutas fue originalmente descrita en el género *Dacus* por Wiedemann, en 1830. La espécie fue transferida posteriormente al género *Anstrepha*. Esa transferencia creo discordancia de género entre las palabras *Anastrepha* (femenina) y *fraterculus* (masculina), lo cual es contra las normas de nomenclatura taxonómica. La concordancia gramatical implicaría el cambio del nombre *fraterculus* por uno totalmente diferente del originalmente propuesto, *sororcula*. Este caso es reglamentado por el Código Internacional de Nomenclatura Zoológica (nombre en aposición). Entretanto, en muchos trabajos publicados antiguamente en el Brasil (p.e. Silva et al. [4]) se encuentra *A. fratercula*, que corresponde a una denominación equivocada de la espécie, una vez que, la palabra *fratercula* no existe en el latin.

La primera discución detallada de la variación morfológica de *A. fraterculus* fue hecha por Stone [5] y como resultado de su estudio, estableció tres nuevas sinonimias y describió dos espécies bastante próximas de la mosca suramericana. Stone hizo dos observaciones validas hasta hoy: (1) *As treated here, it [A. fraterculus] extends from the Rio Grande Valley in Texas south to Argentina, and it is possible that it will eventually be found to represent a complex of species rather than a single one.* (2) *It is probable that several other species will be found in the complex, and further biological work may necessitate an alteration of the concepts given here.*

Las sinonimias de *A. fraterculus* fueron listadas por Zucchi [6], cuando estableció tres nuevas sinonimias.

Anastrepha fraterculus (Wied., 1830)
Dacus fraterculus Wied., 1830
Tephritis mellea Walker, 1837
Trypeta unicolor Loew, 1862
Anthomyia frutalis Weyenbergh, 1874
Anthomyia frutuum, erro
Anastrepha fratercula, erro
Anastrepha soluta Bezzi, 1909
Anastrepha peruviana Townsend, 1913
Anastrepha peruana, erro
Anastrepha distans, Greene, 1934 *nec* Hendel
Anastrepha distincta Greene, 1934 (*partim*)
Anastrepha brasiliensis Greene, 1934

Anastrepha trinidadensis Greene, 1934
Anastrepha suspensa, Lima, 1934 *nec* Loew
Anastrepha costarukmanii Capoor, 1954
Anastrepha scholae Capoor, 1955
Anastrepha pseudofraterculus Cappor, 1995

La mosca suramericana de las frutas ha sido colocada en el grupo *fraterculus*, que reune aproximadamente 30 espécies [7]. En ese grupo están, entre otras espécies, *A. obliqua*, *A. sororcula* y *A. zenildae*, que presentan el ápice del ovipositor muy semejante al de *A. fraterculus*. De un modo general *A. fraterculus*, *A. obliqua*, e *A. sorocula* son las moscas de las frutas más comunes en el Brasil.

La identificación de *Anastrepha* es basada principalmente en el formato del aculeus (ovipositor). Los caracteres de los machos no permiten la identificación específica. Las cuatro espécies antes mencionadas pueden ser separadas, con base en el ápice del acúleo, de la siguiente forma:

a. dientes agudos sobre más de la 1/2 apical (Fig. 5) ....*A. obliqua*
    dientes arredondeados...b
b. dientes sobre más de la mitad apical (Fig. 7) .... *A. zenildae*
    dientes aproximadamente hasta la mitad apical c
c. ápice con 0,17 a 0,19 mm (Fig. 6) .... *A. sororcula*
    ápice con 0,25 a 0,27 mm (Fig. 1 a 4)...................................... *A. fraterculus*

Las espécies de *Anastrepha* de un mismo grupo son separadas en detalles del ápice del acúleo, entretanto, como algunas vezes los limites para la separación no son bien definidos, otras técnicas han sido utilizadas para auxiliar en la identificación:

• Estudio morfométrico multivariado: Con base en las caracteristicas del ápice del acúleo, estan siendo realizadas medidas en ejemplares de *A. fraterculus*, *A. obliqua*, *A. sororcula* y *A. zenildae*. Los resultados preliminares han mostrado que las poblaciones estudiadas pueden ser separadas através de este método. Poblaciones de *A. fraterculus* del sur del Brasil, que presentan un ápice mas largo, son diferentes de poblaciones del nororiente brasileño (Fig. 1-4). La longitud del ápice del acúleo y la distancia entre el fin de la abertura genital y el inicio de la parte denteada fueron las caracteristicas que más influenciaron en la separación de las espécies.

• Citotaxonomía: Apenas algunos trabajos fueron realizados sobre la citogenética de *Anastrepha*. Con relación a las espécies brasileñas, Solferini & Morgante [8] estudiaron los cariótipos de ocho espécies de *Anastrepha*: *barnesi*, *bistrigata*, *fraterculus*, *obliqua*, *pickeli*, *pseudoparallela*, *serpentina* y *striata*. De acuerdo con esos autores, *A. fraterculus* fue la única espécie que no pudo ser identificada con base en la morfología del cromosoma. Fueron encontrados cuatro cariótipos distintos, sugiriendo que podrian representar espécies crípticas.

• Bioquímica: Estudios de sistemática bioquímica han buscado definir mejor los limites para la identificación de *Anastrepha*. Con relación a *A. fraterculus* los resultados han mostrado verdaderas diferencias genéticas entre las poblaciones, debido probablemente a la presencia de espécies crípticas [9, 10].

- Estudio molecular: Las técnicas de DNA están siendo aplicadas a la taxonomía de algunas espécies colectadas en el Brasil [11] y representan una fuente alternativa para la taxonomía de *Anastrepha*.
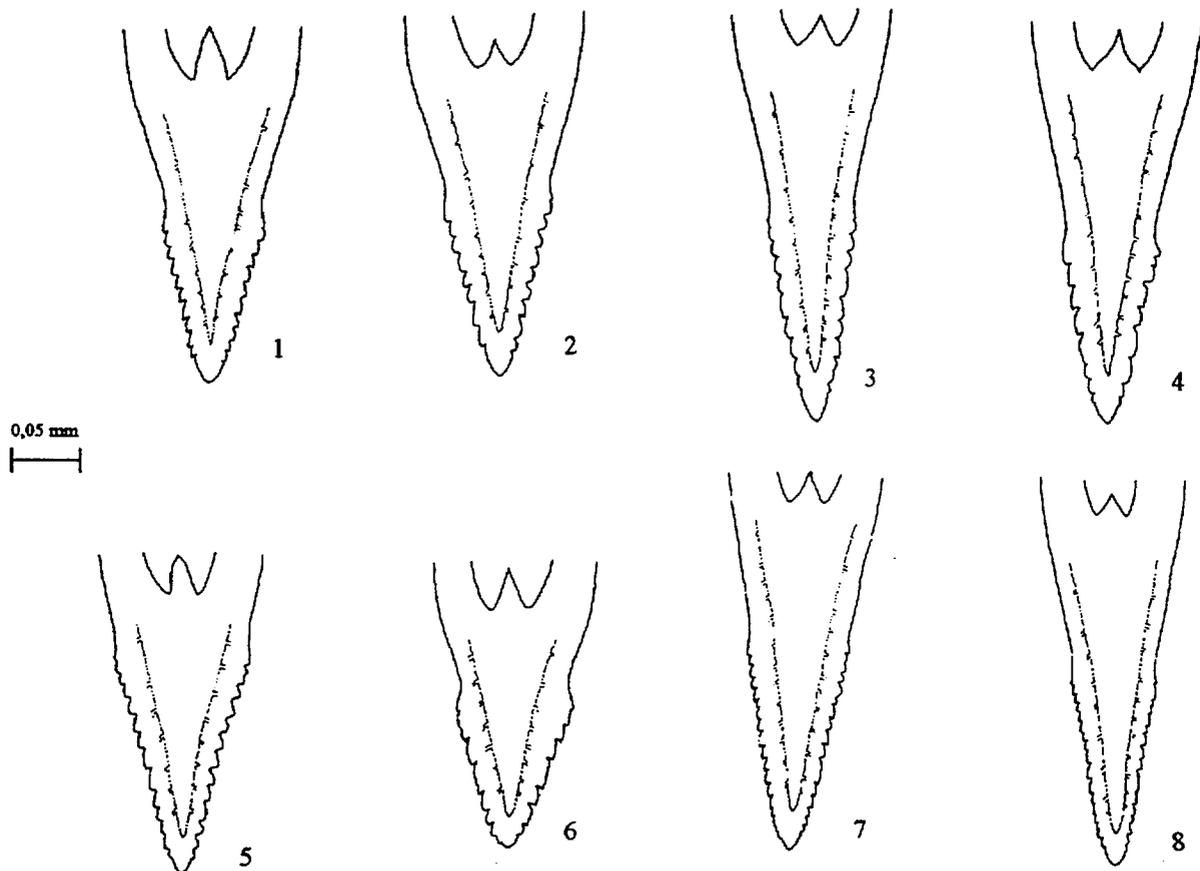
Figuras 1 a 8. Ápices del ovipositor (ventral). 1 a 4 A. fraterculus (1. Rio Grande do Norte; 2. Minas Gerais; 3. Paraná; 4. Rio Grande do Sul); 5. A. obliqua (Pernambuco); 6.A. sororcula (Rio Grande do Norte); 7. A. zenildae (Rio Grande do Norte); 8.A. turpiniae (Mato Grosso do Sul).

3. PLANTAS HOSPEDERAS

El levantamiento más reciente de hospederos de moscas de las frutas en el Brasil fue realizado por Malavasi *et al.*[12], que asociaron *A. fraterculus* con 19 hospederos de várias regiones del país. Los hospederos de la mosca suramericana, en la región de Pelotas (RS), fueron estudiados por Salles[13]. *Anastrepha fraterculus* vuela y ataca frutos, sin distinción, hasta 10 m de altura del suelo [14] y el desarrollo larval y pupal es directamente afectado por el hospedero de al mosca [15].

Las plantas hospederas de las espécies de *Anastrepha* fueron catalogadas por Norrbom & Kim [7]. Para *A. fraterculus* fueron relacionados cerca de 90 hospederos, de los cuales, aproximadamente 30% son referencias para el Brasil. Entretanto, aún existen áreas brasileñas donde nunca fueron realizados levantamientos de moscas de las frutas. Recientemente, por ejemplo, en levantamientos intensivos realizados en el Estado de Goiás, ubicado en la región central del Brasil y con una formación vegetal particular llamada de cerrado, fueron

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descubiertos nuevos hospederos para la mosca suramericana de las frutas, siendo todos ellos nativos de la región [16]: arazá *Psidium australicum* (Myrtaceae), bacupari *Salasia campestris* (Hipocrataceae), cagaita *Eugenia dysenterica* (Myrtaceae), curriola *Pouteria ramiflora* (Sapotaceae). Las informaciones sobre los hospederos nativos de una región son de extrema inportancia, pues estan directamente relacionados al manejo de las moscas de las frutas.

En el Amazonas (Manaus), al norte del Brasil, *A. fraterculus* ha sido colectada apenas en *Terminalia catappa* (Combretaceae) [17]. Esta es una planta ornamental introduzida al Amazonas y es la preferida de la mosca suramericana, apesar de la presencia de otros frutales nativos e introduzidos que normalmente son atacados por esta mosca en otras regiones del Brasil. En el Estado de Mato Grosso do Sul (región suroccidental del Brasil), levantamientos de moscas de las frutas obtenidas de 13 espécies de frutos hospederos, dentro de los cuales algunos conocidos como hospederos de la mosca suramericana e incluyendo *T. catappa*, *A. fraterculus* fue identificada apenas de guayaba *Psidium guajava*. En esta región *A. obliqua*, *A. sororcula*, y *A. zenildae* fueron más abundantes e infestaron más hospederos que la mosca suramericana y en muestras de guayaba fue identificada, además, *A. turpiniae* (Fig. 8), espécie también muy semejante a *A. fraterculus*. Un hecho semejante fue observado en Rio Grande do Norte (región nororiental del país), donde *A. zenildae* fue la espécie más común [18].

Portanto, *A. fraterculus* no es siempre la espécie predominante en los levantamientos realizados. Se debe ser cuidadoso durante la identificación específica de las moscas de las frutas, una vez que las espécies del grupo *fraterculus* de mayor importancia económica son morfologicamente baste semejantes entre sí. Es por esto que antes de intentar explicar el comportamiento de las poblaciones de *A. fraterculus*, es necesario tener seguridad que la identificación específica fue correctamente realizada y mantener *voucher specimens* que puedan ser examinados para el esclarecimiento de eventuales dudas.

4. BRACONÍDEOS PARASITOIDES

Los braconídeos (subfamilia Opiinae) han sido los parasitóides más usados para el control biológico de moscas de las frutas en todo el mundo. Desde que fueron retomados, hace apenas algunos años, los estudios de taxonomía de este grupo de insectos en el Brasil, ha aumentado continuamente el interes por conocer más al respecto de las espécies nativas y su posible uso en el manejo de las moscas de las frutas. Igualmente estos estudios están siendo acompañados por estudios con la espécie introduzida *Diachasmimorpha longicaudata*.

Una revisión sobre los trabajos brasileños con parasitóides de Tephritidae, acompañada de registros inéditos sobre distribución geográfica y moscas hospederas, fue presentada por Leonel Jr. *et al.* [19]. Los parasitóides de tefritídeos en América del Sur fueron discutidos por Zucchi & Canal [20]. Otros parasitóides de *A. fraterculus*, además de braconídeos, fueron discutidos por Salles [13].

Con base en esos trabajos, en el Brasil, *A. fraterculus* es parasitada por seis espécies de braconídeos , siendo cinco de Opiinae y una de Alysiinae. Los opiíneos son *Doryctobracon areolatus*, *D. brasiliensis*, *D. fluminensis*, *Opius bellus* y *Utetes* (*Bracanastrepha*) *anastrephae*; el alisiíneo es *Asobara* sp. *Doryctobracon areolatus* es la espécie más ampliamente distribuida en el país.

El papel real de esos braconídeos en la regulación de las poblaciones de moscas de las frutas es aún desconocido en América del Sur, la verdad es que en la región poca importancia ha sido dada a los estudios con esos parasitóides. La mayoría de las observaciones se refieren a registros de ocurrencia, faltando por tanto, el desarrollo de estudios básicos (biología, comportamiento, dispersión, eficiencia del parasitismo, etc.). Sin embargo, apesar de que poco es conocido de la importancia de las espécies nativas, existen muchas referencias de introducciones de parasitóides de moscas de las frutas para los paises suramericanos.

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TAXONOMIC STATUS OF *Anastrepha fraterculus*

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Abstract

TAXONOMIC STATUS OF *Anastrepha fraterculus*.
There has long been speculation that nominal *Anastrepha fraterculus* comprises more than a single biological species. Herein is a review of data supporting the hypothesis that multiple cryptic species are present. Evidence includes unusual variation in pest status, morphology, karyotypes, isozymes, mitochondrial DNA and cuticular hydrocarbons. The data strongly support the notion of multiple cryptic species. However, it is not yet possible to state how many species may be involved or to delineate them by diagnostic morphology, distribution, host plants or behavior. A combination of methodologies will be needed to resolve the complex.

1. INTRODUCTION

The only comprehensive revision of the genus *Anastrepha* [1] recognized 142 species. An updated key [2] includes most subsequently described species. Additional species have been described [3-12] so that 183 species are now recognized [13-15]. The number of *Anastrepha* species already recognized and the rate at which new ones continue to be added make it clear that much taxonomic work remains to be done on the genus. At least 25 additional undescribed species with clear morphological differences are known [16]. The taxonomy of *Anastrepha* is based mainly on wing pattern and characters of the female genitalia [1]. Many of the known species are morphologically very similar (cryptic) and misidentification is a common problem. Males of many species currently cannot be identified at all [2,17], although recent studies have found characters in the male genitalia that are useful for identification and analysis of phylogenetic relationships [9,18]. The immature stages are poorly studied [19], although third instar larvae can be distinguished in most of the 13 species for which they are known [20].

2. THE *ANASTREPHA FRATERCULUS* COMPLEX

One of the most difficult problems in *Anastrepha* taxonomy involves one of its most important and ubiquitous pest species, the South American fruit fly, *Anastrepha fraterculus* (Wiedemann). Data from a number of sources (variation in isozymes, karyotypes, morphology, and pest status in different areas, as discussed below) indicate that populations now regarded as *A. fraterculus* actually comprise more than one cryptic species. Since Stone's revision [1], these populations have generally been treated as a single, widespread, polyphagous pest species. These populations, henceforth termed the *A. fraterculus* complex, occur from Mexico to Argentina and have been reported to attack about 80 host plants, including major fruit crops such as citrus, mango, guava, and coffee [13].

A clear understanding of the systematics of the complex is necessary for successful application of quarantine and control measures for crop protection. If multiple species with

different distributions, host preferences and behaviors exist, plant protection agencies must be vigilant in preventing the introduction of new pests into uninfested crop areas. Control and eradication methods, especially those based on the sterile insect technique (SIT), must be correctly chosen to suit a biologically and genetically known target, i.e. to ensure that sterile males of the correct cryptic species are released in each area.

2.1. Geographical distribution

The distribution of the *A. fraterculus* complex is much broader than that of any other *Anastrepha* species, with the single exception of *A. obliqua* (Macquart). The complex has a more or less continuous distribution from the Rio Grande Valley of northern Mexico, through eastern Mexico and the Yucatan, all of Central America and into northwestern South America. In Mexico and Central America, populations seem to be concentrated in lowland areas. On the South American continent *A. fraterculus* apparently occurs in two broad, unconnected bands: (1) along the western and northern edges of the continent, including both lowland and montane regions of the Andean countries (Colombia, Ecuador, Peru and Venezuela), and Guyana; and (2) along the east coast of the continent from Fortaleza (northeastern Brazil) to central Argentina; its southern range includes Uruguay, Paraguay and western Argentina. Current knowledge indicates the presence of a large hiatus in the distribution of *A. fraterculus* in the vast middle area comprising the Amazon basin and the hot, dry region separating the basin from the Atlantic coast. The reality or extent of the hiatus is unclear, as *A. fraterculus* has been noted in the Manaus area where it is apparently restricted to tropical almond and mango, both introduced fruits in this region [21]. *Anastrepha fraterculus* also occurs in the Galapagos Islands where it is known to have been introduced [22].

2.2. Hosts and Pest Status

Some authors [23,24] have noted that *A. fraterculus* attacks different hosts in different areas, although this perception does not seem to be fully supported based on a broad compilation of collecting and rearing records [13]. However, the pest status of *A. fraterculus* clearly varies geographically. It is an abundant pest all along the eastern coast of Brazil [25] south to Argentina. In the Venezuelan and Colombian Andes it is the most common and economically important fruit fly species [26,27]. In the Amazon basin, Mexico, Costa Rica, and lowland Venezuela, on the other hand, it is rarely encountered as a pest [21,23,28-31]. Recent studies such as [21,32] have been extremely informative in documenting the distribution and hosts of *A. fraterculus* in non-agricultural areas.

2.3. Morphological Variation

Anastrepha fraterculus displays considerable morphological variability, e.g. in wing pattern, and this explains in part why it has been described under ten different names. In his revision of the genus, Stone [1] took a conservative approach and considered the various forms to be simply geographical races of a single species. He readily admitted, however, that "in this part of the genus there is the greatest difficulty in determining specific limits" and that several other species would probably be found in the complex. Others [23] have taken exception to Stone's classification and considered the Mexican form of *A. fraterculus* to be a distinct species based on host plant relationships and slight morphological differences. However, no one has been able to provide a diagnosis to distinguish consistently the Mexican form, nor has it been formally named. *Anastrepha fraterculus* varies in thoracic coloration,

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including the size of a dark spot on the scuto-scutellar suture [16]. In specimens from the central Andes the spot is generally larger, such that Peruvian specimens have been misidentified as *A. suspensa* (Loew) [33], which occurs only in the Greater Antilles, Bahamas, and Florida. There is also variation in the length of the aculeus and the shape of its tip, but there seems to be overlap among small samples of the populations that have been studied [16]. A broad geographical analysis of samples tested for other characters has not yet been attempted.

2.4. Karyotypes

The karyotype of Mexican *A. fraterculus* [24] differs from Brazilian specimens [34]. Four other distinguishable karyotypes have been ascribed to an assortment of *A. fraterculus* populations in Brazil [35]. This magnitude of karyotype variation does not occur in most other *Anastrepha* species and strongly suggests the presence of cryptic species. Unfortunately, data were not available in [34, 35] to correlate the various karyotypes with delimited areas, host plants, morphology, isozyme patterns or other information useful in characterizing species.

2.5. Isozyme variation

The first large survey of isozyme variation in *Anastrepha* [36] compared 15 species; included were 16 different samples of *A. fraterculus* from various fruits and areas of Brazil. From this study, the authors concluded that as many as four different taxa might be included among their *A. fraterculus* samples. However, they were unable to provide any means of characterizing these taxa and urged caution in setting species boundaries. Subsequent studies [37,38] presented evidence that there is not a simple relationship between these putative species and their host plants as in *Rhagoletis*.

Another isozyme study [39] specifically addressed the taxonomy of *A. fraterculus* in terms of its biogeography. Geographical areas inhabited by morphologically variable forms of *A. fraterculus* throughout its range were included. As a standard of reference, equivalent data were included for three other species of *Anastrepha* collected over the same geographical range: these were *A. obliqua* (Macquart), *A. distincta* Greene and *A. striata* Schiner. The results showed strong genetic differentiation within nominal *A. fraterculus* which is probably due to the presence of cryptic species. Extreme frequency and/or fixed allele differences were found among samples from Andean vs. lowland Venezuela, and southern Brazil vs. Bahia. Genetic differences among *A. fraterculus* populations were far larger than any observed among populations of the reference species. The *A. fraterculus* complex as it now stands may not even be monophyletic.

There is also intriguing allozyme variation that suggests the presence of two cryptic species at Itaquera, São Paulo state, Brazil [39]. This is an historically important *A. fraterculus* site where numerous studies on population genetics, behavior and ecology of *A. fraterculus* have been conducted [40-44]. Interestingly, two karyotypes have also been described from Itaquera [35]. If, in fact, two cryptic species are present, there are profound implications for the interpretation of published information from this site. Careful population genetic analysis is necessary to clarify this situation.

2.6. Mitochondrial DNA variation

Mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) has also been reported in *A. fraterculus* [45]. Three of the four samples analyzed were from the same series of populations that showed strong genetic differentiation of isozymes. Substantial mtDNA variation was observed; indeed, all four populations (lowland and Andean Venezuela, Bahia and southern Brazil) could be uniquely identified based on RFLP patterns of three restriction endonucleases. Results strongly corroborated isozyme data indicating lack of gene flow between contiguous lowland and montane regions of Venezuela. However, the data were inadequate to further clarify relationships among more broadly distributed geographic populations. A finer and more continuous scale of geographic sampling, i.e. along transects, is needed to establish the presence or absence of clines and distinct genetic boundaries.

2.7. Cuticular Hydrocarbons

Differences in cuticular hydrocarbon (CHC) composition among species has proven useful in discriminating fruit fly adults and larvae [46]. A CHC analysis of the same samples used in the isozyme analysis of [39] revealed that the population from lowland Venezuela has a homogenous CHC pattern that is distinguishable from all others; the remaining samples could not be distinguished from each other [47].

2.8. Laboratory Crosses

A series of laboratory crosses was performed based on differences found in and among *A. fraterculus* populations in Brazil by a combination of techniques including isozyme, karyotype and morphological analysis [48]. Egg hatching rates and offspring sex ratios showed clear deviations from expected values in crosses between 'type I', 'type II', and 'morphotype CSS' flies indicating various degrees of reproductive isolation probably at a species level. The types are at least partially sympatric, although types II and CSS are predominately in coastal regions, and they share at least some hosts such as guava.

3. CONCLUSIONS

Data from several genetic and biochemical analyses, especially karyotype and isozyme studies, strongly support the hypothesis that more than one cryptic species are represented under the current concept of *A. fraterculus*. Two such species probably occur in Venezuela: a pest species in montane regions, and a non-pest in lowland areas. Two or more additional species probably occur in Brazil. Relationships among these populations and those in other Andean countries have not been examined. Geographical features such as the Andes mountains, the Amazon basin, and the drylands of interior Brazil may provide effective geographical barriers to gene flow. It is unclear exactly how many species may be involved and whether it is possible to delineate all of them by diagnostic morphology, genetics, distribution, host plants or behavior.

Observations on fruit fly pests such as *A. fraterculus* tend to focus on cultivated areas and hosts, hence there is surely much that we don't know about its current and pre-historic distribution. For example, *A. fraterculus* may have only a limited, and perhaps recent, occupation in the Amazon basin. It is possible that pest populations of *A. fraterculus* have been spread in historical times far beyond their original areas of endemism.

4. SUGGESTIONS FOR FUTURE RESEARCH

There are immense gaps in our knowledge of the taxonomy and biology of *Anastrepha* in general and the *A. fraterculus* complex in particular. The size of the genus and its distribution over large portions of biologically poorly known regions of South and Central America are direct causes.

Coordinated input from many sources will be required to resolve the *A. fraterculus* complex. Before the putative species can be characterized biologically and geographically, and their potential as pests determined, taxonomic methods to distinguish them must be developed. The following are crucial needs to resolve the taxonomy of the complex:

- Conduct surveys along transects: from coastal to montane to Amazon basin in the Andean countries; from north to south in coastal Brazil; and from the coast to the interior of Brazil and Argentina.
- Compile host rearing records and seasonal phenology of populations along transects.
- Conduct multiple analyses on all samples (morphology, karyotype, isozyme, DNA, cuticular hydrocarbons). The technology exists to do multiple analyses on **single specimens**. It remains to be learned whether karyotype, isozyme and mtDNA markers sort together or independently.
- Conduct surveys in non-agricultural areas to determine the overall distribution and feral hosts.
- Establish laboratory colonies for studies of rearing, behavior, genetics, and ability of different populations to interbreed.

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KARYOTYPE STUDY OF THE SOUTH AMERICAN FRUIT FLY,
Anastrepha fraterculus (Wied.) IN ARGENTINA

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Abstract

KARYOTYPE STUDY OF THE SOUTH AMERICAN FRUIT FLY, *Anastrepha fraterculus* (Wied.) IN ARGENTINA.

The most frequent karyotype of *Anastrepha fraterculus* in Argentina is described here on the basis of mitotic metaphase morphology. It was named "*fraterculus Arg 1*". The diploid number is 2n = 10 + XX/XY and in males it comprises five homomorphic pairs and one heteromorphic pair, the latter being the sexual pair. Samples from different populations were cytologically analyzed, and "*fraterculus Arg 1*" is present in all of them at a high frequency (about 60 %). A typical C band pattern of the X chromosome was found only in the Montecarlo (Misiones province) population.

1. INTRODUCTION

The genus *Anastrepha* is scarcely known from a taxonomic point of view, and species identification is based on the morphology of the apex of the ovipositor [1]. Male adults and inmatures are mostly undistinguishable. The most serious taxonomic problem involves *A. fraterculus* because intraspecific variation is not well understood.

Cytological studies of Brazilian [2] and Mexican populations [3] of *Anastrepha fraterculus* have demonstrated karyotypic variation. Bush [3] indicated that it was not possible to identify karyotypical differences among *A. fraterculus*, *A. mombinpraeoptans* and *A. distincta*.

Solferini and Morgante [4] investigated samples collected on various species of host fruits from different areas of Brazil. They distinguished four karyotypes, which differed in their sexual chromosomes. Karyotype 1 presented a Y/X ratio of approximately 0.5 ; C banding produced a heterochromatic block on each end of the X chromosome and a totally heterochromatic Y chromosome. Karyotype 2 differed from the former in having a longer Y chromosome, carrying a satellite on it. Karyotype 3 had a long X chromosome with a constriction which separated 1/3 of the distal portion of the chromosome; the Y chromosome being similar to that of karyotype 2. In karyotype 4, both sex chromosomes had a similar length and a secondary constriction. This work suggests that *A. fraterculus* represents a complex of sibling species. Through further investigation, karyotype 3 was later assigned to *A. sororcula* [5].

A great amount of chromosomal variation within Argentine populations of *Anastrepha fraterculus* has been detected [6] & [7].

The present work reports the existence of the reference (most frequent) karyotype (59,7%) within regional samples of *Anastrepha fraterculus* as well as a configuration which, up to now, would be a marker of Montecarlo population.

2. MATERIALS

Larvae were obtained from green and yellow fruits of guava shrubs, probably *Feijoa sellowiana* (from Castelar and Ituzaingó, Buenos Aires province) and *Psidium guayaba* (from Ituzaingó, Buenos Aires province and Tucumán province) and from *Prunus persica* collected from Montecarlo, Misiones province*. Samples of adult flies were systematically identified as *Anastrepha fraterculus* (courtesy Eng. Norma Vaccaro, INTA, Concordia).

Third instar larvae were recovered (n=210) from host-fruit pulp to obtain cytological preparations.

3. METHODS

Mitotic metaphase plates were prepared from neuroblast cells of third instar larvae. Reproductive tissue was obtained from adults one day after emergence.

3.1. Preparation of cerebral ganglia.

Larvae were dissected in a drop of 0,75 M KCl. Each cerebral ganglion was put separately on a culture slide with KCl during 5 min, changed to fresh fixing solution (1:3, glacial acetic acid, ethanol) for 6 min, and then to 45% acetic acid for 3 min. Each ganglion was transferred to a microscope slide with a drop of 45% acetic acid and squashed by striking it with a glass or plastic rod, to spread the tissue. Slides were air dried horizontally and kept in closed coplin jars for at least 15 days at room temperature. The same treatment was used for reproductive tissue.

In both cases the tissue was stained in 4% orcein solution and then squashed in lacto-propionic medium (1:1), to obtain slides.

C banding was carried out as described in Ref.[8], with the following modifications: bariumhydroxide solution was used at 27-29°C for 7 min. Chromosomes were stained with 5% Giemsa (Gurr R66) solution in phosphate buffer (pH 6.8) for 15 min.

3.2. Preparations were examined without mounting them.

A drop of immersion oil was put directly on the slide. Such preparations can be kept for six months after examination.

4. RESULTS

Karyotypical analysis showed the existence of chromosomal diversity among and within Argentine geographic populations.

The most frequent karyotype (59,7%), present within all the analyzed samples was named "*fraterculus* Arg 1" (from now on "*f*Arg 1"). It is the reference wild type karyotype for

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* Buenos Aires = Central region; Tucumán = North Western region; Misiones = North Eastern region.

Argentina. Its 2x = 10 + XX/XY complement, consists of five pairs of homomorphic and telocentric autosomes, an acrocentric   X chromosome and a small submetacentric Y chromosome   (Fig. 1). The Y chromosome is approximately 2/3 the length of the X chromosome. The autosomal pairs are almost undistinguishable one from each other, except for pair II which is characteristically the largest in the complement: X/II = 0.74. The X chromosome is generally curve shaped

It is worthwhile explaining that in populational studies the heteromorphic pair is generally associated with the sexual pair. However, in *fArg 1* this was not the case, because the same heteromorphism was observed in meiosis plates.

The C banding technique applied to *fArg 1* has revealed two opposite terminal blocks of heterochromatin on the X chromosome, one near the centromere and the other one can be marking a secondary constriction. From now on, it will be named "X_1". The small submetacentric Y chromosome showed a pericentromeric C band (Y_1). These are useful markers to identify *fArg 1* (Fig.1).

In addition to *fArg 1*, other less frequent chromosomal configurations might be shared by two or more *A. fraterculus* Argentine populations. This is also the case for the sample of peaches collected from Montecarlo, Misiones. Nevertheless the chromosomal analysis also demonstrates the existence of a distinctive karyotype. The Y_1 chromosome is approximately 1/2 the X chromosome and the ratio X/II = 0.82. C banding technique, showed that the X chromosomes carry two bands on one end (Figure 2). This particular banding of the sexual chromosome (named "X_2") was only observed among flies of the Misiones population, thus it can be considered as a marker.
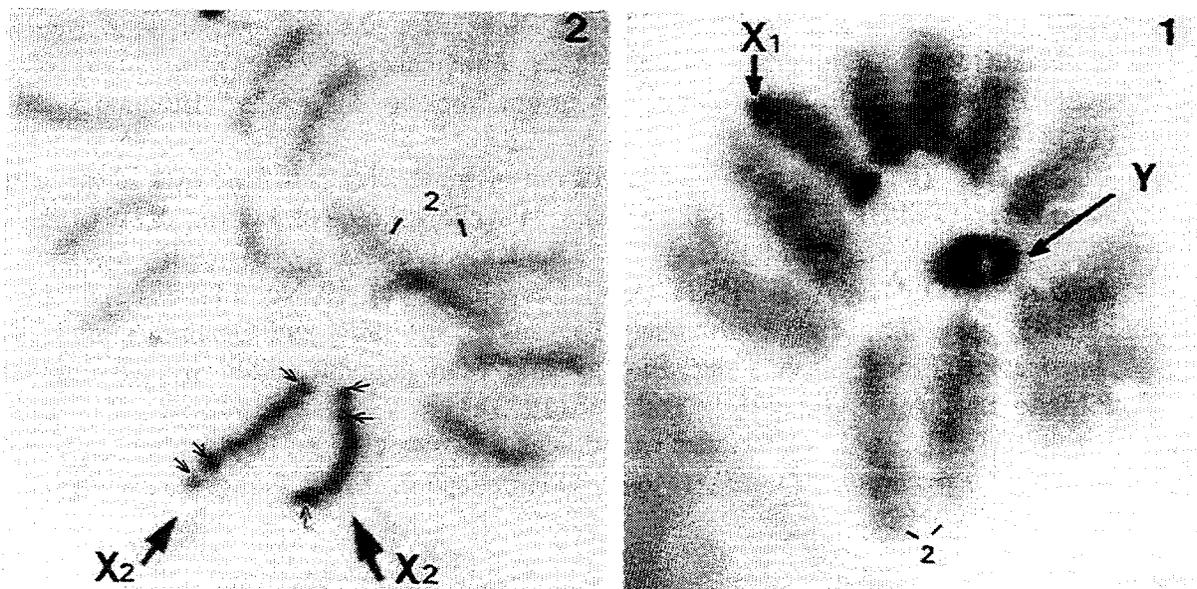
Figure 1 and 2:   *(1) Mitotic metaphase of male "fraterculus Arg 1" carrying the X_1; C banding reaction. (2) Mitotic metaphase of female from Montecarlo, Misiones carrying the X_2; C banding reaction.*

5. DISCUSSION

Cytogenetic studies of *A. fraterculus* revealed that *fArg 1* is different from the karyotypes previously described by Solferini and Morgante.[4] for Brazilian populations of this insect. As discussed by Bush [3], no heteromorphism of the sexual pair was observed in Mexican populations, whereas in Brazilian specimens a heteromorphic sex pair was detected [2].

The X_1 chromosome showed the same C banding pattern as that of "karyotype 1" described by Solferini and Morgante.[4], which carries a totally heterochromatic Y-chromosome and presents a Y/X ratio of 0,5, thus differing from *fArg 1*.

The X_2 chromosome of Montecarlo, Misiones is longer than the X_1: it has an extra C band. These data are correlated with the differential ratios between Y_1/X_1 and Y_1/X_2, and between X_1/II and X_2/II.

Investigation of "*Anastrepha fraterculus complex*" needs the work and support of different groups and technologies so that results can be correlated in views to understand phylogenetic relationships.

The effort of cytologists in applying rigorous scientific techniques to visualize chromosomal differences which are probably markers of reproductivelly isolated populations will be helpful in taxonomic studies. Cytological data are a tool which supplements and strengthens the work of taxonomists based on morphological data.

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BREEDING TECHNIQUE OF *Anastrepha fraterculus* (Wied.) FOR GENETIC STUDIES

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Abstract

BREEDING TECHNIQUE OF *Anastrepha fraterculus* (Wied.) FOR GENETIC STUDIES.
Various samples of Anastrepha fraterculus from different areas in Argentina were obtained to develop artificial breeding in the laboratory. Based on a modification of Salles's method, an improved artificial rearing of the species was developed with satisfactory results for genetic analysis. The advances made will contribute towards the search for genetic mechanisms for control.

1. INTRODUCTION

Argentinian orchard fruits are attacked by pests commonly named "fruit flies", in reference to insects of the *Ceratitis capitata* (Wied) species and of the *Anastrepha* (Schiner) genus. Both groups cause problems to the country forbidding access to foreign commerce.

While *Ceratitis capitata* or Mediterranean fruit fly is an accidentally introduced species that has spread over subtropical and moderate world regions, the genus *Anastrepha* is native and is restricted to the American continent.

Blanchard [1] described 36 species of the genus *Anastrepha* for Argentina, based, in some cases, on very small samples. Our laboratory received samples coming from several areas of the country. Only specimens of *A. fraterculus* (Wied) could be identified by Miss Norma Vaccaro, responsible for the systematic determination of flies samples all over the country. Nevertheless, Vattuone et al. [2] described for the locality of Andalgalá, province of Catamarca, based on a few specimens, the presence of other species: *A. dissimilis* (Stone) and *A. alveatoides* (Blanchard).

Taxonomic work made on the genus *Anastrepha* has been mainly based on ovipositor morphology and wing morphology, an approach that restricts the analysis to only one sex.

Cytological studies have been done on Mexican populations [3] and Brazilian populations [4] in order to recognize different species, finding divergences between classical systematic and cytological data. These studies revealed the presence of caryotypical variants within the species and similar cytological morphologies between different species.

Analysis of *Anastrepha* populations showing biochemical, morphological and cytological variants, with different hosts and geographic distribution, some of them performed together [5][4][6], have not clarified the problem. Moreover, the situation in the case of *A. fraterculus*, was suggested to be a complex of cryptic species.

The management of this pest in the laboratory gives a skillful tool to make possible genetic studies in views to explain the described complexity. The first attempts that were made for the artificial breeding of *A. fraterculus* were carried out in Peru by Gonzalez et al. [7]

and Gonzalez [8]. Later, in Brazil, Salles [9] presented another methodology for rearing the species.

A modification of Salles's method has been developed with satisfactory results in our laboratory.

2.1. Results

There are important and particular steps so as to develop the artificial rearing of the species. However, there are fundamental and general patterns to consider in the artificial management of this pest. In natural conditions, the larvae and eggs of this species occur inside the fruits, where water is not a restrictive factor for their development, because the fruit is commonly hydrated. Also, although there are not exact data about the influence of humidity on the pupal stage, our experience shows that this stage can be modified by relative humidity differences.

The following prospectus can be established, considering the previous basis:

2.1.1. Adult recovery

Infested fruit samples from the country were received and were in the larval and pupal stage of the insect. The recovery of adults required controlled humidity conditions. Recovering of imagos from pupae at environmental humidity conditions was 25.64 ±.16% while at controlled humidity conditions of 85% RH was 69.42±0.9%.

2.1.2. Obtaining eggs

Recovered adults were put in 3-litre-capacity bottle with plastic froth caps which make air exchange possible, and were grouped in mates or pools.

The adult food consisted of a diet composed by 2 parts of brown sugar and 1 part of corn hydrolyzed protein (R.M. SAIC 1). It is obtained by heating the sugar in a double boiler up to its total dissolution and then adding the hydrolyzed protein to the dissolved sugar. We put it in small and deep containers. With a net over the containers we diminish the possibility of the flies remaining stuck on the sugar when they eat. If humidity conditions are enough to allow this mixture to remain humid, it is changed once a week to avoid infections.

Oviposition substrates (agar fruits) are composed of half spheres prepared with 2% agar in hot water up to its dissolution, when 0.2% of red dye is added (amaranto in hydroalcoholic solution to 0.3%). This mixture is poured over the internal face of half pingpong balls and allowed to harden at room temperature. It is wrapped with ParafilmR stretched to maximum and it is conserved in a freezer, to avoid dehydration.

These agar fruits are put on is base with its convexity up and they are put off in 48 hours. Females lay the eggs inside that agar substratum (in our experience, oviposition is produced only in the zones covered by ParafilmR but not in zones exposed by the rupture of that membrane). In the conditions of our laboratory, oviposition substrates are offered for 48 hours. The number of eggs laid per female having 1 to 3 agar fruits was tasked as weekly recollected in that 48 hours lapse, from families composed by one male and one female with different ecological origins is shown in Table 1.

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Table 1. NUMBER OF EGGS LAID PER FEMALE HAVING 1 TO 3 AGAR FRUITS AVAILABLE (STOCK 284).

CONCEPT	1 agar fruit		2 agar fruit		3 agar fruit	
	Per fem.	Per Fruit	Per Fem.	Per Fruit	Per fem.	Per Fruit
1 COUPLE/BOTTLE	35.5±1.29	35.5±1.29	37.5±4.20	25±2.10	56.5±2.82	20.4±1.79
5 COUPLE/BOTTLE	13.1±0.63	13.1±0.63	14.8±0.88	8.4±0.6	31.9±1.4	10.6±0.8

It can be observed that the increment of total oviposition is associated to the number of available fruits; nevertheless the possibility of distributing the eggs on several fruits causes a decrease of eggs per fruit.

However, when these substrates were put in bottles containing populations that presented a greater number of females, the quantity of eggs collected over each agar fruit increased up to 169.21 ± 0.19 average eggs.

Egg recovery from the substrate is carried out using an entomological needle and putting the eggs all together on a Petri dish with distilled water. The recovered eggs are put in 40 c.c. vials filled with water up to one third of its capacity and they are closed up. These vials must be agitated for 48 hours in a Roller bottle agitator at 25°C and 6 rpm. Once this period has passed, eggs are drained off on a filter paper, which is placed over the larval food.

Percentage of hatching eggs must be measured after 48 hours of agitation, but a problem is created because some eggs hatch during this period and then the technique efficiency diminishes. In Table 2, egg-hatching percentages for families of different origins are shown.

Table 2. FERTILITY OF DIFFERENT ORIGIN FAMILIES MEASURED THROUGH EGG ECLOSION.

Origin	Number of families	Egg fertility %	Range in %/ family
A	9	42.4±0.5	7-66
B	7	66.9±0.5	48-78
C	20	69.9±0.2	19-94

2.1.3. Larval feeding

The larval period in tephritids is very important for their development. They reserve the nutrients that give the necessary energy not only for surviving, growth and development at this stage, but also the resources that larvae will utilize for the great metamorphosis which takes place at the pupal stage and for the energy transference to the adult stage.

Table 3. DATE OF PUPAE PERCENTAGE TAKEN FROM EGGS NUMBER

Origin	Number of families	Pupae/eggs %	Range in %/family
B	12	25	0-52
C	2	24	11-40
D	7	59	4-100

Table 4: NECESSARY TIMES FOR THE BREEDING PHASE OF *ANASTREPHA FRATERCULUS*

```
Biological    cycle  obtained  by  the  descripted
                        protocol
Temperature 24° C  | 24° C  |      25° C      |      25° C      |    25° C

Day    |————————|————————|————————————|—|—|————————————|—|—|————————|
       0          2        4           18 20 24           31 33 35      43

Stage  |    E  G| G      |  L A R V A E  |P  U  P  A  E  | First    | Last |
       (embrionic        |              (first pupaes    imagae     imagae
          development)    |               and larvae
                          |               isolation)

Environ Oviposi- |destilated  Larvae diet        Vermiculite
ment    tion     |water and
        sustrate |agitador
```

Several diets, which have already been described in the previously mentioned works or in others, have been tried, but the best results were obtained with the following formula:

1 kg boiled carrot + 1 kg raw carrot (both pulverized)
400 g white sugar
600 g corn flour
150 g powder of dry ferment (Calsa 2)
6 g Sodium benzoate
1 g methyl p-hydroxibenzoate NipaginR* dissolved in alcohol.
Adjusted with hydrochloric acid to P.h. 4.5

Everything is homogeneized with a mixer. This food is put in containers, according to egg number, in an approximate proportion of 200 eggs over 200 g of food. They are isolated in containers with a vermiculite base, closed up with paper so as to permit the whole explotaition of environmental humidity. In the breeding conditions (25°C and 85% RH) the first pupae complete development after 18 days. At that time, it is useful to separate the rest of the larvae from the food and put them on a vermiculite base to help pupation.

Comparative data for percentage of pupae recovered relative to egg number are shown in Table 3.

Table 4 has condensed the necessary times for each breeding phase of *A. fraterculus* following this technique.

Although this technique can be improved, it represents the beginning of *A. fraterculus* breeding for its genetic analysis. Genetic studies need a management through individual couples making possible, for instance, cytological studies of karyotypical compatible complements. These advances will allow us to explain some previously determined complexities in the species about natural populations, and will allow to induce variability and rearrangements to begin with the search of genetic mechanisms for control.

ACKNOWLEDGEMENTS

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ARTIFICAL REARING OF *Anastrepha fraterculus* AND OTHER ANASTREPHA SPECIES

PRELIMINARY STUDIES FOR THE COLONIZATION OF
Anastrepha obliqua AND *Anastrepha serpentina* (DIPTERA: TEPHRITIDAE)

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Abstract

PRELIMINARY STUDIES FOR THE COLONIZATION OF *Anastrepha obliqua* AND *Anastrepha serpentina* (DIPTERA: TEPHRITIDAE).

A series of trials were carried out with the aim of collecting preliminary data for the colonization of *Anastrepha obliqua* and *Anastrepha serpentina*. Trials were focused on evaluating adequate oviposition media as well as their effect on fly demographic parameters; the effect of cage population densities on demographic parameters was also considered; for *A. serpentina*, egg disinfection treatments by organic acids was assayed, and a screening study was carried out for suitable pupation media. Nylon-made egging mesh resulted in the most efficient oviposition medium, while low insect densities provided the best conditions for increased rates of fly fertility. Organic acids (methyl-p-hydroxy-benzoic) were found to hamper egg hatch, while a variety of pupation media provided improved fly emergence rates vs. the naked pupation method.

1. INTRODUCTION

Increased economic importance of the fruit flies for the agricultural scenario of Latin American has prompted consideration for Integrated Pest Management methodologies for these pests (Aluja & Liedo, 1986). The *Anastrepha* spp. complex is among the most important fruit flies that affect the Mexican cash crop farming; in the frame of a fruit fly national program (SARH, 1988), a project has therefore begun in Mexico that was devoted to establish procedures for the colonization of these insect species, as well as to develop practical techniques for the artificial mass rearing of their biological stages.

Such studies would provide basic information on applied research issues, such as the application of genetic control measures to *Anastrepha* spp., post-harvest treatments, etc.

This paper contains preliminary information on (a) the evaluation of different oviposition media and (b) studies on egg hatchability, that were all carried out on colonized specimens of *A. obliqua* and *A. serpentina*; of the latter species, two studies were also devoted to evaluate the effect of disinfection treatments by organic acids on egg hatch and to evaluate the performance of several pupation media.

2. MATERIALS AND METHODS

For all colonization trials, wild insects were employed that had originated from larvae and/or pupae field-collected from fruits. Fruits were recovered from commercial groves in the Soconusco area of Chiapas State, Mexico.

Fruits used for specimen collections included: mango fruits (*Mangifera indica*) for recovering *A. obliqua* specimens and mammel apple fruits (*Mammea americana*) for recovering *A. serpentina*. During this research, it was noticed that individual *A. fraterculus* flies could be also easily collected from guava (*Psidium guajava*) fruits for rearing studies. Collected fruits were confined in plastic boxes and kept in a room at 26 ± 1°C and ca. 85% RH to allow larval maturation and pupation. Larvae were feeding in fruits until they reached the third instar, when they emerged to pupate in sterilized soil that was placed in the rearing boxes below fruits.

Adult flies that emerged from pupae were artificially fed on a 1:3 ratio of sucrose and hydrolyzed protein (ICN Biochemicals ™), in wooden framed cages that were located in a room kept at 26 ± 1°C and 70-75% RH; an artificial light photoperiod was maintained that was of 14:10 (light: darkness) prior to studies.

2.1. Evaluation of six oviposition media for *A. serpentina* and *A. obliqua*

To compare different oviposition media for these species, 30 *A. serpentina* or 10 *A. obliqua* adult flies that had reached sexual maturation (ca. 10 days after emergence) were used. These were confined in cylindrical transparent plastic cages (13.5 cm diameter and 10 cm high for *A. obliqua*; 15 cm diameter and 17.5 cm high for *A. serpentina*), and their oviposition rate were assessed over a 30 day period. Sex ratio of insects inside cages was 1:1.

At one side of each cage, a round portion of net (15 cm^2 surface) was applied to serve as the oviposition substrate, and 15 cm large plastic box, filled with ca. 3 ml of water, served for egg collection.

In these trials, six oviposition media were tested: three consisted of natural cotton nets, with different oviposition hole sizes (22, 48 and 57 holes/cm^2); one consisted of a plastic mosquito mesh (58; wide filter cloth part No. SP4058; 3,200 holes/cm^2); one consisted of nylon mesh, that was completely filled; the last treatment consisted of a curine-made medium, also completely filled. This was tested only for *A. serpentina* specimens.

For all treatments, the following parameters were considered: (1) mean number of eggs collected from the water reservoir of the cage every 24 hours; (2) mean number of eggs laid into mesh and collected there, by the use of a thin brush; (3) mean number of collected eggs that hatched; (3) survival of male and female individuals, after 30 days of colonization; (4) fecundity and fertility rates of both species (considered over 50 days of colonization); (5) rate of reproductive decrement; and (6) loss of reproductivity of the two species. Each treatment was replicated three times, data were analyzed by the X^2 means separation test (p=.05).

2.2. Evaluation of egg hatching of colonized *A. serpentina* under different cage population densities

In this test, the effect of adult overcrowding in cages on egg hatching rates was tested. For this purpose, glass 30 x 30 x 30 cm cages were used, which contained 50, 150, 550, 1,350 or 3,850 sexually mature adult insects. Aproximately 3 hours after emergence, flies were transfered into cages by the use of an entomological aspirator.

As oviposition media, agar-agar, spherical media were used, as described by Berrigan et al. (1988). The number of oviposition spheres was positively related to the different insect density treatments to maintain a constant relationship between the number of flies inhabiting the cage and the total surface available for oviposition. Therefore, treatments were designed as follows: 50/sphere; 150/sphere; 550/sphere; 1,350/ sphere and 3,850/sphere.

Spheres were removed and replaced each 24 hours, and eggs were extracted from the medium by means of a dissection needle, then placed in 15 cm diameter Petri dishes in lots of 100 per dish. Petri dishes were maintained in an incubation room at 26 ± 1°C and 75% RH before determining the rate of hatching (%), which was assessed after 72 hours of incubation. Data were analyzed by means of the X^2 test at P = 0.05.

2.3. Evaluation of egg hatching of colonized *A. serpentina* as related to egg-disinfection by an organic acid

The effect of methyl-p-hydroxy benzoic acid (sodium salt) at a 0.03% (w/w) dose on egg hatch of *A. serpentina* was evaluated. To this end, five samples of 400 eggs collected in agar-agar spherical media were prepared by treatment in a 0.03% (w/w) methyl-p-hydroxy benzoic acid water solution. Temperature for treatment was kept at 26 ± 1°C ; treatment lasted 72 hours.

The percentage of hatched eggs obtained from this treatment was compared with those provided by four samples of 100 eggs each, treated in sanitized (hypochlorite-treated) tap water.

After treatment, eggs were placed in Petri dishes and maintained in an incubation room (same as 3.2), prior to assessing hatch after rinsing in potable tap water. Petri dishes were kept in an incubation room at 26 ± 1°C and 75% RH, prior to determining egg hatching assessed at the completion of 120 hours of incubation. Data were analyzed by means of the X^2 test at P = 0.05.

2.4. Evaluation of seven pupation media for *A. serpentina*

In these trials we evaluated, as pupation substrates for *A. serpentina*, the fitness of vermiculite, a vermiculite-sand combination, a vermiculite-soil combination, pure sand, a sand soil combination, organic soil and naked pupation occurring over a plastic suface. When combinations of pupation media were considered, these were made at 1:1 ratios.

Each pupation medium was tested in Petri dishes, maintaining ca. 2.5 cm depth of pupation medium. All Petri dishes held 10 third instar larvae. Flies were assessed by measuring their emergence rate (%) after pupation, which were therefore retained as a measure of the fitness of each medium for oviposition purposes. Data were analyzed by means of the X^2 test at P = .05.

3. RESULTS AND DISCUSSION

3.1. Evaluation of six oviposition media for *A. serpentina* and *A. obliqua*

As shown in Table 1, the nylon mesh was the egging medium that produced the highest oviposition activity in both insects, providing the highest efficiency as oviposition medium in

terms of number of eggs collected from the water reservoir, for both *A. obliqua* and *A. serpentina* species; for the latter, it also was the most efficient in retaining eggs in the net (average 820.3 and 812.3, respectively).

In the study related to *A. serpentina*, the cotton (57 holes) mesh also provided significant number of egg either by water collection (average 295.3) or by mesh collection (average 156.6), and it was challenged by the plastic mosquito mesh, in the case of egg water collection (avg. 120.6).

In the case of mesh collections in *A. obliqua*, the nylon mesh (226.3) was challenged by the only plastic mosquito mesh (44.3). The nylon mesh provides the best option as egging medium for both species: it should be noticed that, in the *A. serpentina* case, either an oviposition system based on water-collection or mesh-collection, similar to *A. ludens*, can be considered. Data on survival rates of male and female individuals of both *Anastrepha* species indicate that treatments had equivalent effects: Table 2 (a) and Table 3 (a).

In the same tables is shown that treatments had no effect on hatchability of *A. obliqua* eggs, while the nylon mesh resulted in significantly enhanced egg hatchability of *A serpentina*, followed by the cotton mesh (57 holes/cm^2).

Table 1. AVERAGE NUMBER OF EGGS COLLECTED FROM DIFFERENT OVIPOSITION MEDIA IN TWO SPECIES OF *ANASTREPHA* (SCHINER), AFTER 30 DAYS OF OBSERVATION.

Treatment		A. obliqua		A. serpentina	
Type	Holes/cm^2	Avg. No. of eggs		Avg. No. of eggs	
		(mesh)	(water)	(mesh)	(water)
Nylon	*	226.3a	7.3a	812.3a	820.3a
Mosquito	3200	44.3b	13.6a	48.0c	120.6c
Cotton	48	4.3c	11.0a	5.6d	38.0d
Cotton	57	3.3c	3.6a	156.6b	295.3b
Cotton	22	0.0	12.0a	5.6d	41.6d
Curine	*	*	*	51.3c	14.6e

* Complete filled

Each treatment was replicated three times. Data indicated by the same letter are not significantly different after X^2 analysis (P = .05).

Demographic analysis data of Tables 2(b), 2(c) 3 (b) and 3 (c) show that gross and net insect fecundity rates were positively influenced by the nylon and by the mosquito mesh in the case of *A. obliqua*, while in the case of *A. serpentina* the best performance relates to the nylon and to the cotton mesh (57 holes/cm^2). Only *A. serpentina* confirmed this pattern for fertility rates, it is possible that data of *A. obliqua* depend on its oviposition behavior or on egg handling during collection.

Table 2(a). NUMBER OF SURVIVING INSECTS AND NUMBER OF EGGS OBTAINED FROM DIFFERENT OVIPOSITION MEDIA IN *Anastrepha obliqua* (Shiner), AFTER 50 DAYS OF OBSERVATIONS.

Treatment			A. obliqua		
Mesh type	Holes/sq cm^2	# insects /cage (1:1)	Number survived		Number of Flies Emerged
			Female	Male	
Nylon	*	10	46.6a	26.6a	7
Mosquito	3200	10	20.0a	33.3a	0
Cotton	48	10	26.6a	26.6a	0
Cotton	57	10	13.3a	13.3a	0
Cotton	22	10	13.3a	20.0a	0

* Completely filled
Values followed by the same letter are not significantly different (X^2 test, P = .05)

Table 2(b). FECUNDITY AND FERTILITY DATA ASSOCIATED TO DIFFERENT OVIPOSITION MEDIA IN *A. OBLIQUA* (50 DAYS OBSERVATIONS).

Treatment		Gross rates (a)		Net rates (a)	
Mesh type	Holes/c m^2	Fecundity %	Fertility %	Fecundity %	Fertility %
Nylon	*	87.26	0	47.26	0
Mosquito	3200	16.10	0	6.86	0
Cotton	48	6.3	0	3.79	0
Cotton	57	4.2	0	1.36	0
Cotton	22	6.67	0	4.79	0

* Complete filled

Table 2(c). CAUSAL FACTORS ASSOCIATED TO DECREASED RATES OF FECUNDITY AND FERTILITY IN *A. OBLIQUA* FOR DIFFERENT OVIPOSITION MEDIA (50 DAYS OBSERVATION).

Treatment		Causal factors for loss of reproductive rate		
Mesh type	Holes/cm^2	Hatch %	Mortality %	Interact %
Nylon	*	100	45.8	100
Mosquito	3200	100	57.4	100
Cotton	48	100	39.9	100
Cotton	57	100	67.7	100
Cotton	22	100	28.2	100

* Completely filled

Table 3(a). NUMBER OF SURVIVING INSECTS AND NUMBER OF EGGS OBTAINED FROM DIFFERENT OVIPOSITION MEDIA IN *ANASTREPHA SERPENTINA* (SHINER), AFTER 50 DAYS OBSERVATION.

Treatment			A. serpentina		
Mesh type	Holes/sq cm^2	No. insects/ cage (1:1)	Number survived		No. of Flies Emerged from eggs
			Female	Male	
Nylon	*	30	75.6a	71.1a	179
Mosquito	3200	30	77.8a	62.2a	17c
Cotton	48	30	68.9a	77.8a	8c
Cotton	57	30	77.8a	64.5a	72b
Cotton	22	30	77.8a	71.1a	0
Curine	*	30	53.3a	44.4a	2c

Completely filled
Values followed by the same letter are not significantly diferrent (X^2 test, P = .05).

Table 3(b). FECUNDITY AND FERTILITY DATA ASSOCIATED TO DIFFERENT OVIPOSITION MEDIA *IN A. SERPENTINA*, AFTER 50 DAYS OBSERVATIONS.

Treatment		Gross rates (a)		Net rates (a)	
Mesh type	Holes/cm^2	Fecundity %	Fertility %	Fecundity %	Fertility %
Nylon	*	57.13	8.75	56.63	8.44
Mosquito	3200	6.58	0.17	6.57	0.16
Cotton	48	1.70	0	0.97	0
Cotton	57	22.02	1.30	20.32	1.20
Cotton	22	2.86	0.07	2.42	0
Curine	*	3.47	0	2.8	0

* Completely filled

Table 3(c). CAUSAL FACTORS ASSOCIATED TO DECREASED RATES OF FECUNDITY AND FERTILITY IN *A. SERPENTINA* FOR DIFFERENT OVIPOSITION MEDIA, AFTER 50 DAYS OBSERVATION.

Treatment		Causal factors for loss of reproductive rate		
Mesh type	Holes/cm^2	Hatch %	Mortality %	Interact %
Nylon	*	84.7	0.90	85.30
Mosquito	3200	97.5	0.20	97.60
Cotton	48	100	42.9	100
Cotton	57	94.01	7.8	94.60
Cotton	22	97.00	15.40	98.80
Curine	*	100	19.40	100

* Completely filled

Table 4. EFFECT OF DIFFERENT CAGE POPULATION DENSITIES ON EGG HATCHABILITY OF *Anastrepha serpentina*.

Treatment sq. cm/fly	Mean hatch (%)
108	36.36a
36	36.10a
12	31.90a
4	19.80ab
2	12.10b

(Data followed by the same letter are not significantly different).

Table 5 (a): EFFECT OF A 0.03% (W/W) WATER SOLUTION OF METHYL-PARA-HYDROXY BENZOATE (NIPAGIN TM) ON EGG HATCHABILITY OF *Anastrepha serpentina*.

Blocks	Methyl-p-hydroxy benzoate					Yap water				
	% hatch per replicate				total	% hatch per replicate				total
	1	2	3	4		1	2	3	4	
I	4	7	6	10	27	13	9	16	16	54
II	2	0	2	2	6	1	4	1		6
III	1	1	2	1	4	0	0	5	1	6
IV	0	0	0	0	0	0	1	0	1	2
V	0	0	0	0	0	2	6	0	2	10

Table 5 (b): X^2 ANALYSIS ON HATCHABILITY PERCENT VALUES IN *Anastrepha serpentina*.

Treatment	% Observ. hatch (O)	% Expect. hatch (E)	(O-E)	-E/E =x
Methyl para hydroxy--benzoate (.03% w/W)	38.0	58.0	-20.0	6.897
Tap water	78.0	58.0	20.0	6.897
Total	116.0	116.0	0.0	13.794

3.2. Evaluation of egg hatchability of colonized *A. serpentina* under different cage population densities

Results shown in Table 4 are related to the influence of adult cage-pupulation densities and clearly indicate that the best conditions for artificial rearing were found in micro-environments with decreased of insect densities, i.e. 12, 36 and 108 cm³/fly providing the best conditions to the newly colonized insect. Data were submitted to X^2 test (P = .05).

Table 6.   *Anastrepha serpentina* RATE OF LARVAE-ADULT FLY
RECOVERY COMPARED IN SEVEN DIFFERENT PUPATION MEDIA.

Treatment test	Number emerged adult flies	Larvae to adult recovery (%)	X sq.
Soil (organic)	162	90.00	a
Sand & soil	135	75.00	a
Sand	133	73.00	a
Vermiculite & Soil	133	72.00	a
Vermiculite	117	65.00	a
Vermiculite & Sand	105	58.30	b
Naked pupation	9	5.00	c

3.3. Evaluation of egg hatchability of colonized *A. serpentina* as related to egg disinfection by an organic acid

The effect of methyl-para-hydroxy benzoic acid on hatchability of *A. serpentina* eggs was found to hamper egg hatch, possibly due to the permeability of the chorion to acid diffusion: Tables 5 (a) and 5 (b). Data were submitted to X^2 test (P = .05).

3.4. Evaluation of seven pupation media for *A. serpentina*

Data on larva-to-adult recovery (% emergence) as related to pupation media indicate that organic ground, the mixed sand-ground, sand, the mixed vermiculite-ground, and vermiculite are equivalent pupation media, superior to both the vermiculite-sand mix and to naked pupation. Data submitted to X^2 at P = .05 (Table 6).

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MASS REARING OF THE MEXICAN FRUIT FLY, *Anastrepha ludens,* AT THE FRUIT FLIES BIOFACTORY IN METAPA DE DOMINGUEZ, CHIAPAS, MEXICO

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Abstract

MASS REARING OF THE MEXICAN FRUIT FLY, *Anastrepha ludens,* AT THE FRUIT FLIES BIOFACTORY IN METAPA DE DOMINGUEZ, CHIAPAS, MEXICO.

A description of the present methods for mass rearing *Anastrepha ludens,* known as the Mexican fruit fly, at the Fruit Flies Biofactory in Metapa de Dominguez, Chiapas, is given. Important contributions and improvements are described for the rearing stages, e.g. egg production and incubation, larvae diets, lab conditions for the development of larvae and pupae, larvae and pupae handling and environmental control.

1. INTRODUCTION

As main part of the Mexico National Campaign against Fruit Flies, a Fruit Flies Production Biofactory was built in Metapa de Dominguez, Chiapas, Mexico, to produce flies in the genus *Anastrepha.* This biofactory was designed based on the experience obtained from Mediterranean fruit fly (*Ceratitis capitata,* Wied.) mass rearing for 14 years, transfer of lab technical aspects from the Mexican fruit fly mass rearing in Mission, Texas, USA, as well as technology developed by the Methods Development Department of the Mexico-Medfly Program.

The Biofactory was built with three production modules, two for fruit fly mass rearing and a third for parasitoids (*Diachasmimorpha longicuadata*). Each module has the capacity for producing 150 million individuals and at present is used for *Anastrepha ludens* and *A. obliqua* production. Besides these species, in the future it is intended to mass rear, in each module, *A. striata* and *A. serpentina,* species which are of the most economic importance in Mexico and included within the eradication strategies of the Campaign in the different Mexican states.

Goals of the Campaign are based in the success reached in mass production of *Anastrepha* spp. Applying the Sterile Insect Technique (SIT) combined with integrated control strategies in each specific area of the country.

This paper describes the methods used at present for mass rearing. *A. ludens,* taking into account that these methods are those which have worked through a series of previous evaluations.

2. HANDLING THE COLONY

The colony is one of the main pillars of insect mass rearing, since it depends totally on the insect adjustment capacity for reproduction in an artificial environment. The values or parameters used to assess the efficiency of the colony are egg fecundity and fertility.

The colony is kept confined in Mission type aluminum cages with the following dimensions: 2.15 × 1.80 × 0.30 m, covered by a mesh made of glass fiber. The oviposition panel is on the front and center of the cage and consists of linen fabric to simulate the host fruit surface where flies lay eggs. The fabric is covered with a silicon thin layer to obtain a soft surface to make easy egg collection. It is also within a chamber with aluminum walls and door made of acrylic which isolate eggs from the external environment to avoid egg desiccation.

Eggs are collected once a day in the morning (it starts at 7:00 a.m.) with the use of spray nozzle which sprays water on the panel, and eggs slip to the inner part of the cage where they are collected in a container; later, they are disinfected with chlorine at 200 ppm for 10 minutes; then they are placed in plastic decanters with a determined egg volume and sent to the incubation area.

After eggs are collected, the oviposition panels are disinfected with chlorine at 2,800 ppm to allow a new oviposition period in an environment free of microorganisms. Once oviposition starts and the excess moisture has decreased in the oviposition fabric, a very thin spray of gel fusselerone is applied. This gel is in a pressurized container and applied with a fan pattern nozzle. This procedure is repeated in the afternoon to assure that eggs remain in a hydrated medium and will not lose viability to dehydration.

An acceptable production is of 2×10^6 eggs per cage per day with 90% fertility. These parameters, considered as a minimum, are reached with a colony completely adjusted to be handled in artificial conditions, whereas there is a temperature of $27 \pm 1°$ C and a relative humidity of 50-60%, which have been determined as ideal, as well as a photoperiod of 14:10 hours (light:darkness).

The use of these methods has evolved according to the problems faced. First of all the oviposition panel, which was made of plastic material called *curine* which, besides its high cost, needed to be perforated, since it has a plastic cover to ease the introduction of the ovipositor. Thus, linen is an alternative which works more efficiently and is practical to use.

Humidity injection is used to avoid egg dehydration within the oviposition chambers using ultrasonic dehumidifiers, which proved to be effective when handling fewer cages in the colony. The problem was when the colony increased to obtain a greater production and the oviposition chambers did not retain 100% of the humidity injected. For this reason, the relative humidity percentage in the environment increased up to 80%, causing low fly longevity, general contamination and loss of productivity. The use of the hydrating gel in a low concentration has solved such problems, and has assured an egg production with the desired quality.

Fly density inside the cages is another factor related to egg production efficiency and its quality. At present, a density of 150,000 flies per cage produce from 1 to 2 million eggs per cage, per day, in fly's productive life. Flies reach production peaks from the fifth to the tenth day. The tendency is to decrease fly populations per cage to a level of 110,000 individuals, which may produce similar amounts to those produced by 150,000 flies per cage, of acceptable quality, with better handling conditions and environment with less stress (Figure 1).
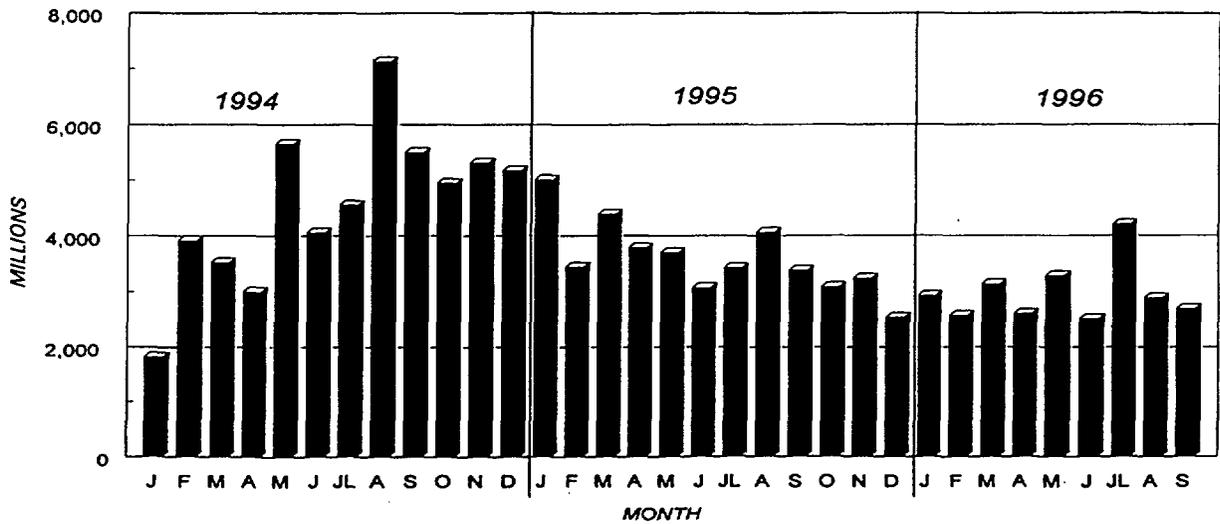
Figure 1. Egg production.

The handling of temperature is another factor that directly influences colony production. If the temperature is less than 26°C, sexual activity also diminishes (less matings) and, at the same time, the production index decreases. If the temperature is greater than 28°C, there is an increase in sexual activity and a greater egg production but longevity or productive life of the fly diminishes seriously. At present temperature inside the colony area has been kept between 26 and 28°C, and most of the time it remains between 27 and 28°C, a fact that has favoured the reduction of sexual maturity time from 10 days, which was considered normal for many years, to eight days, the value accepted at present. This helps greatly to make productive life of flies greater than 12 days, for a total of 22 days of confinement in the cage, with eggs since the first day of oviposition with 90% fertility.

3. EGG INCUBATION

After collection, disinfected eggs are placed in polycarbonated 8 liter plastic bottles to which 240 ml of eggs plus, 4,800 ml of water are added to get an egg:water ratio of 1:20, Water is previously purified by microbiological filters and Ozone action to diminish the possibility of contamination of the egg that may affect its viability. Egg incubation takes place after four days at an environmental temperature of 27°C and from 23 to 26°C inside the bottles. The incubation water is changed daily to avoid microbial population increases and, at the same time, at 48 hours of incubation, eggs are disinfected again with 5ppm of chlorine within the same bottle for 10 minutes. Water is eliminated by decantation and clean water is added at 2 ppm of chlorine to allow incubation to continue.

Eggs inside the bottle are kept oxygenated with pressurized air (20psi), which makes them remain in a continuous movement, avoids sedimentation and allows homogeneous incubation conditions.

Each ml of eggs contains approximately 21,000 eggs. Inside each bottle around five million eggs are incubated.

After four days of incubation eggs are ready to be seeded in the diet and the ratio of water:egg is modified to 10:1. Water used for seeding is mixed with gel (carragenine) to increase viscosity and water density. In this way eggs remain suspended until they are seeded in the diet, which is described as follows.

4. LARVAL DIET

The dream of any fruit fly breeder is to have a single food that may be recommended for several fruit fly species in any region of the world. Obviously, this is not yet possible due mainly to poor knowledge of nutritional requirements of these insects and because of conditions of the region where the work is carried out, which implies poor availability of ingredients, storage difficulties (shelf-life) and environmental problems among others.

Larval diet used at present is made with corn cob particles, corn flour (which works as a texturizer) to give body and volume to diet mixture; dried inactivated yeast (*Saccharomyces cervisiae*) as main source of aminoacids, the vitamin B complex and minerals; white sugar as a carbohydrate source; microbial inhibitors such as methyl-parabene (Nipagin) which has a greater action against fungus and yeast and, to a lesser degree, on gram-negative bacteria; sodium benzotate which acts to a greater degree on yeast and bacteria and to a lesser degree on fungus; citric acid is used to give acidity to larval food and acts in a direct way in microbial control since it creates an inadequate environment for growth of fungus and some bacteria; guar gum as stabilizer to avoid the sineresis effect or loss or non retention of moisture among diet particles; and finally water, which is the universal vehicle to disperse nutrients in the diet, makes up the target percentage in the formula (Table I).

Larval diet is prepared in belt mixers with a capacity of 3.5 tons each, following this mixing procedure:

Texturizers are placed in the mixer, as well as yeast and sugar, which are mixed dry for 10 minutes; then water, citric acid and diluted sodium benzotate are added. Methyl-paraben is left in hot water one day before to facilitate its dissociation since it is not 100% water soluble. The total mixture is homogenized for 30 minutes until it reaches the consistency and texture of the diet. The mixing process is monitored by the Quality Control Department to verify that diet humidity oscillates between 62 and 63% at a pH of 4.3 to 4.4. If it is not, the food will have to be adjusted to these parameters before it is sent to the diet reception area.

TABLE I. INGREDIENTS USED FOR LARVAL DIET

Ingredient	%
Corn cob particles	17.2
Corn flour	5.3
Sugar	9.2
Yeast	7.0
Guar gum	0.1
Nipagin (Metil-paraben)	0.2
Sodium benzoate	0.4
Citric acid	0.6
Water	60.0

All ingredients must fulfill specification requirements determined by the Quality Control Department for larval consumption, which are determined by evaluation and acceptance regulations. A rejected ingredient is not used in mass rearing since it would bring serious consequences and risk for production or deterioration of quality needed by the biological product.

Substantial changes were made in the larval diet formula this year basically to the use of quar gum which solved a problem of high larval mortality since it formed a water film in depressions of the food once placed in larval trays. Such depressions cause mortality of newly hatched larvae since they stayed trapped under the water and cannot breathe. Guar gum has the property of trapping water molecules and not releasing them; this fact avoided these troubles.

Preservatives have changed in concentration as microbiological problems have arisen which affected development and quality of the insect; most of the cases are derived from environmental control troubles.

5. LARVAL DIET RECEPTION

Prepared food is moved to the diet reception area where it is placed, weighted, and distributed in glass fiber trays previously disinfected. Seven kilograms of diet are placed in each tray, then it is distributed and the surface is flattened with a metallic plate to avoid the formation of crests on the diet since these get dry and eggs become dehydrated if are placed on such crests.

Trays are seeded with four ml of eggs suspended in the gel solution. Each trays contains approximately 84,000 eggs with 10 to 20% of hatched larvae to assure that the eggs have reached complete maturity.

The gel used for seeding helps to keep the egg moist until the moment of hatching, which has led to better egg viability.

A density of four ml of eggs per tray was considered suitable after evaluating densities of 3, 3.5, 4, 4.5 and 5ml. Which results in an acceptable larval transformation, but affected size and weight, which must vary between 25 and 27 milligrams.

Trays already seeded are piled in amounts of 30 trays on metallic platforms and moved to the larval starting room prior to this, the platforms were completely covered with a fabric called "pañalina"(kind of gauze used for diapers) to avoid introduction of *Drosophilla melanogaster* (vinegar fly).

6. LARVAL DEVELOPMENT

Larvae have three instars; for this reason, within the rearing process there are three different environments in three different and independent rooms, for nine days of development until they reach maturity.

TABLE II. TEMPERATURE AND RELATIVE HUMIDITY REQUIRED IN EACH DEVELOPMENTAL STAGE IN MASS REARING A. *ludens*

Room	Temperature (°C)	Relative humidity (%)
Colony	27 ± 1	55 ± 5
Incubation	27 ± 1	
Larval starting room	28 ± 1	85 ± 5
Larvae I	27 ± 1	75 ± 5
Larvae II	26 ± 1	75 ± 5
Pupation	21 ± 1	60 ± 5
Pupal maturation	24 ± 1	60 ± 5
Packing	16 ± 1	60 ± 5

6.1. Larval starting room

In this room environmental conditions are established with a temperature of 28 ± 1°C and a relative humidity of 85 ± 5% with the purpose of accelerating egg hatching to avoid dehydration and also to obtain homogeneous larval development.

Newly hatched larvae are kept under these conditions for three days and then moved to the Larvae I room.

6.2. Larvae I room

When larvae end the first instar they are placed in an environment with a temperature of 27 ± 5%. In this room, larvae complete the second instar; the fabric that covers the trays is taken off since this instar generates too much metabolic heat. First instar larvae remain on the diet surface feeding with nutrients dissolved in superficial water since their oral organs are not yet developed.

6.3. Larvae II room

At this place temperature and relative humidity temperature and relative humidity oscillate between 26 + 1°C and 75 ± 5%, respectively. Larvae are located from the middle depth of the diet to the bottom of the tray, looking for more humidity and nutrients available. At this stage the most metabolic heat is generated; temperature in the room diminishes since humidity gets lost gradually at the diet surface and as the third instar advances, the diet changes texture to a fine powder. If the loss of humidity in the diet is very extreme, on the seventh day of development a spray of water with benzoate is applied (1g/liter) on the food surface rehydrate it. When the ninth day of larval development is completed, larvae are a creamy color, the digestive system vanishes from sight and it is considered that it is mature, ready to be separated from the diet.

The remarkable changes in larval development have demanded the division of developmental instars in independent areas to provide ideal conditions for each larva. The result of this change is that the period of 10 days of larval development was shortened to nine days, getting in this way an optimization of the rooms for a greater production capacity (Figure 2).
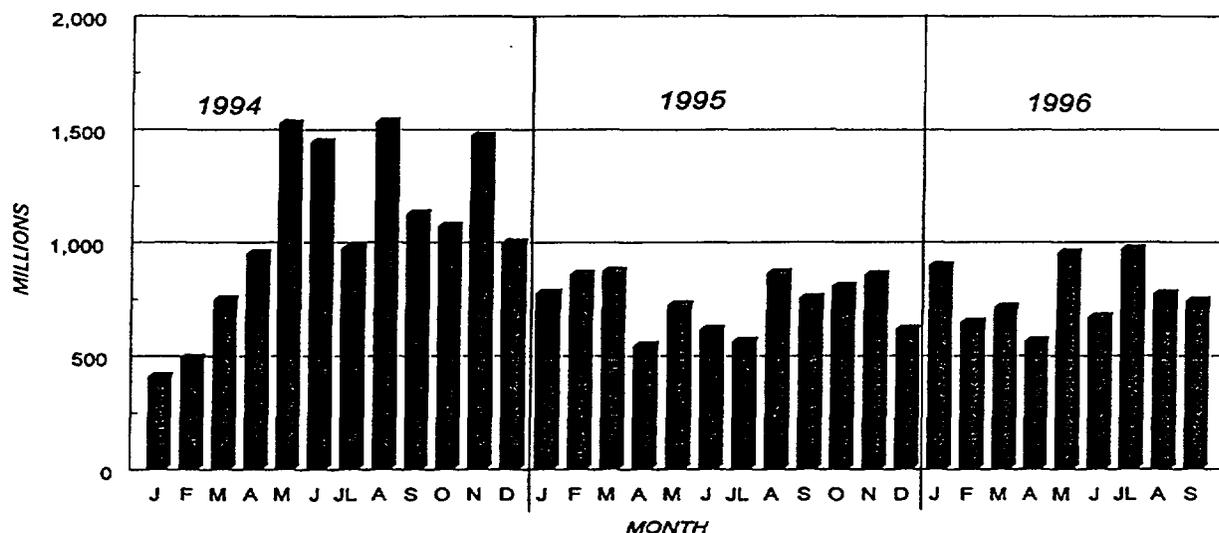
Figure 2. Larvae production.

7. LARVAL SEPARATION

When larvae are completely mature they are moved to the larval separation room, where the necessary equipment is available to separate then from the diet. The equipment consists of a reservoir made of glass fiber and a suction system which works by Venturi effect. The diet from 180 trays is emptied gradually into the reservoir. Trays are then cleaned in a washing machine where they are disinfected with chlorine and then returned to the diet reception area.

When the loading of the reservoir is completed, diet diluted in water is sucked through a plastic pipe in the bottom part of the reservoir; this pipe is connected to another plastic pipe which carries clean water through any intersection This produces a vacuum that sucks the mixture contained in the reservoir (Venturi effect). The mixture of clean water and diet is received in sieves that trap the larvae and release the diluted diet, which drops to a special pipe that carries to a collector tank out of the plant.

There is a dry method for larval-diet separation that uses tombolas. The diet is removed from the trays and emptied in the tombola to break compaction. Tombolas move circularly and the larvae also move within the tombola's sieve in an intermittent way. Larvae passes through the mesh when they are separated from the diet and fall into a container.

Separated larvae are placed in trays with a mesh bottom to drain the excess water, and later they are placed in aluminum trays, also with mesh in the bottom. To these trays, vermiculite is added as population substrate in amounts of one liter of larvae per two liters of vermiculite. Then trays are placed in shelves and moved to the pupation room.

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8. PUPATION ROOM

This room is also called the dark room since it is kept in darkness because larvae are attracted by light and may come out of the trays. Temperature in this room is kept at 21 ± 1°C to induce larvae to be less active. Relative humidity is at 60 ± 5% to decrease the excess environmental humidity when humid larvae enter into this room; this fact delays pupation. Four dehumidifiers are used to keep humidity low, which assures the established levels.

Pupae are kept in this room for three days until they reach a minimum of 85% pupation. Later, the shelves are moved to the pupal maturation room.

9. PUPAE MATURATION AND PACKING

Pupal maturation ends at the eleventh day after finishing pupation, at a temperature of 24 ± 1°C and a relative humidity of 70 to 80%. Maturity is detected by eye coloration of immature stages, which marks the time to be irradiated (two days before emerging adults). At this time the puape are separated from vermiculite by means of a wind tunnel where vermiculite is deposited at the end of the tunnel; the bad quality pupae drop in the center and the good weight puape (minimum 17mg) are dropped at the beginning of the tunnel. In this way the biological material is selected to be released or sent to the colony.

Some years ago the separation of pupae from vermiculite was made inside a tombola with mesh 12 and 10 but it was detected that this caused mechanical damage to the pupae, decreasing their quality. The tunnel has the necessary characteristics for a better handling of the biological material since it uses a pedestal fan at low speed which pushes larvae that drop from a hopper in the top part of the tunnel to the bottom toward the inner part of it. Inner walls of the tunnel are covered with foam to lower the drop of the pupae.

Once pupae have been separated from vermiculite, they are colored with a fluorescent dye (Day Glo^{MR}). The process of painting pupae has the purpose of allowing a differentiation of the sterile flies released in the field from the wild (fertile) ones.
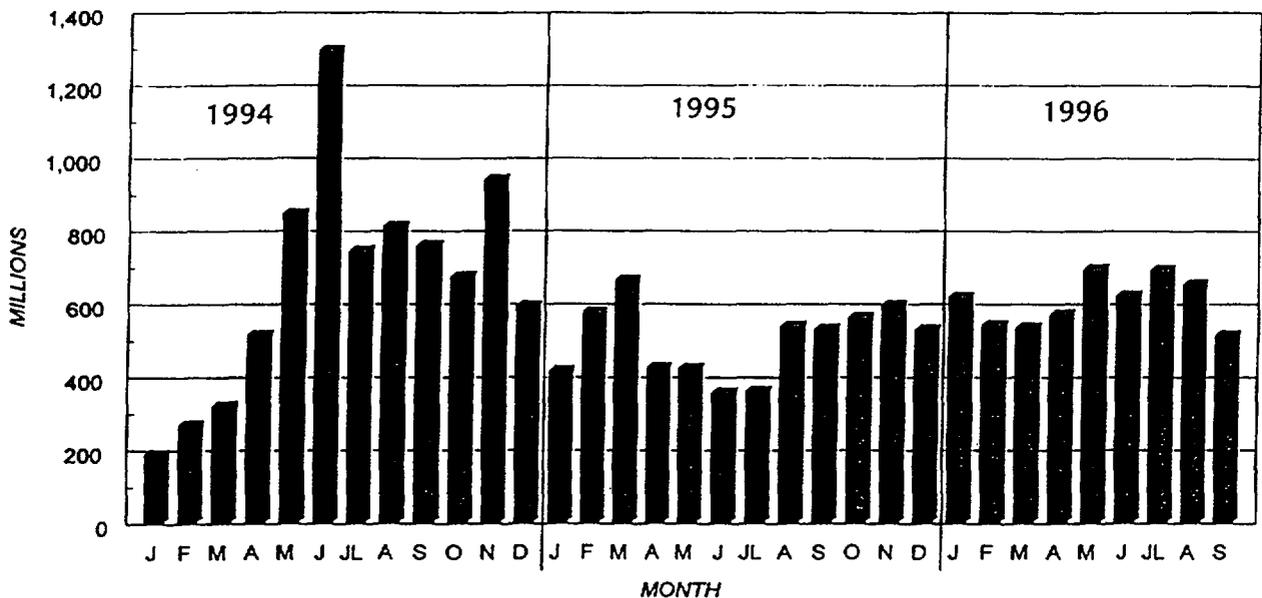
Figure 3. Pupae production.

Painted pupae are placed in sausage type bags to be packed in cardboard boxes in amounts of nine sausages per box. Each box contains 1.2 million pupae, approximately, ready to be irradiated.

Pupae remains one hour in hypoxia within the closed sausages to make ionizing radiation more effective. Each box has a radiosensitive film adhered to detect and certify that the material was adequately irradiated at eight Krads, when the film turns into a dark color.

Biological material is transported to the airport to be sent to the different states of the Mexican Republic, where pupae are packed in No. 20 paper bags and left in emergence rooms to be released in the field later (Figure 3).

10. ENVIRONMENTAL CONTROL OF THE PROCESS

The control of the environmental conditions is, undoubtedly, the most important factor of the mass rearing process. For this reason the lab was designed with double equipment in each room in a way that if one is out of order the other is ready to start working any time.

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PROCEDURES FOR MASS REARING THE WEST INDIAN FRUIT FLY, *Anastrepha obliqua*

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Abstract

PROCEDURES FOR MASS REARING THE WEST INDIAN FRUIT FLY, *Anastrepha obliqua*.

A series of bioassays resulted in a promising colony of *Anastrepha obliqua* (Macquart) for artificial production. A new model circular cage has been designed to achieve an increase in density of adults per cages, thus resulting in an increase in egg production. A bioassay for best egg production as well as constant hydration of eggs until collection time was chosen.. Cotton fabric gave the best results of the fabrics used in the oviposition panel. A new diet based on corn cob particles and with citrus acid instead of hydrochloric acid was tested and showed promising results in good production, quality and less risk in handling. The optimum humidity range for larva to pupa conversion was found to be 70-80%.

1. INTRODUCTION

With the purpose of eliminating restrictions to export mango, citrus caducipholious, guava and other non-traditional tropical commodities, as well as to eliminate direct damages to fruit production, the Mexico National Campaign against Fruit Flies was undertaken. This campaign let to the construction of a plant to produce insects at a massive level, in order to apply the Sterile Insect Technique (SIT) combined with integrated control strategies to reach control of fruit flies of economic importance, with the active participation of fruit growers and State governments.

Among the species of economic importance that the Fruit Flies Complex will produce a massive level is *Anastrepha obliqua* (Macquart). At present a promising colony is available for artificial production, after a series of bioassays for its adjustment process. This paper describes the procedures and advances reached in the rearing process of this species.

2. COLONY

2.1. Cages

At present the breeding colony is handled in Mission type cages with dimensions of 2.15 m (height) x 1.8 m (width) x 0.3m (depth). The front and rear of the cage are covered with a mesh made of glass fiber of 49 holes per square centimeter, except the center of the cage where the oviposition device is located which is called oviposition panel. Each cage houses 55,000 pupae and, with 90% emergence, it is possible to obtain an average of 0.5 adults per square centimeter. This density has allowed us to increase egg production to our present level in spite of the recommendation of Moreno [4] that the maximum density per cage must not exceed 0.2 adults per square centimeter in cages of 30 x 30 x 30 cm (5400 cm^2).

Since the beginning of mass rearing, a new model circular cage has been designed with the purpose of reaching an increase in density of adults per cage and a better egg production. Light intensity required for copulation under lab conditions will also be determined as a behaviour parameter prior to the oviposition period to increase breeding efficiency.

2.2. Oviposition panel

The oviposition panel has the following dimensions 1.5m (height) x 0.7m (wide) x 0.2m (depth). Searching for substrates that fulfill the needs of handling in a massive way, several bioassays were conducted to choose one with the greatest perspective for egg production which, at the same time, allows constant hydration of eggs until collection time.

The first of the assessed substrates was parafilm (American Company™) which although at the beginning was feasible for egg production has the disadvantage that, in order to keep eggs hydrated, it is necessary to use an ultrasonic humidifier in a permanent way; this is an electronic device not manufactured for this specific purpose but if it fails there is a direct effect on egg quality. On the other hand, this is a very fragile substratum which after 5 days in use (the average of egg production is from 12 to 15 days) tends to break down easily and needs to be totally replaced, which is not suitable.

Several types of synthetic fabrics were later assessed (commercially known as *organza, chiffon*, etc.) with 50 to 60 holes per lineal centimeter. A thin layer of silicon was applied to these fabrics on both sides and once placed in the panel some applications of fussellerone at 2% were added to keep eggs hydrated. Results of these assessments were not encouraging because of a lack of adherence of the fussellerone since the fabric was too thin; besides, flies preferred fabrics with fewer numbers of holes per lineal centimeter, which at the same time makes easier handling.

Later, the Research Laboratories in Welsaco, Texas, USA, reported that after several replicates they found a fabric with 16 to 18 holes per linear centimeter showed a better response to the oviposition behaviour in domes with natural red color paraffin (Moreno, D.S. 1994. Personal communication). However, this way of obtaining eggs was not operative in mass rearing processes; for this reason only the number of selected fabrics was evaluated. The result was a cotton fabric with 18 holes per linear centimeter, adapted to the vertical panel in the Mission type cages, which produced encouraging results and surpassed those obtained with other fabrics.

Figure 1. Egg production.

This fabric is called "*tuzor*" and is coated with a thin layer of silicon both sides, which allowed a uniform adherence of the fusselerone applied over it later. This kept the eggs hydrated. Finally, considering that most of the fruit flies present different responses to color attraction, and since yellow and green are the best attractants for *A. ludens*, [6] in *A. obliqua* the response of the adult to green, yellow, white and clear green was assessed. The best results in egg production were obtained on the dark green panel (Figure 1).

2.3. Luminosity

At the beginning and end of the photoperiod a low light intensity is used to favour mating behaviour. This is made by reducing gradually the number of fluorescent lights until a range of 30-40 candles is obtained. Once oviposition starts, light intensity is directed to the oviposition panel in a range of 70-80 candles.

2.4. Photoperiod

During the colonization stage, breeders were kept with light for 24 hours but at the beginning of the mass rearing, the photoperiod was modified to 10 hours of light and 14 hours of darkness.

3. EGG INCUBATION

To determine the adequate process for egg incubation, several methods were evaluated.

3.1. Petri dishes

First, eggs are placed on a humid mesh within the Petri dishes which are also placed inside containers made of styrofoam in order to keep a temperature of 26-27°C to favor hatching at the seeding time. However, since these containers are not hermetically sealed , dehydration of eggs was continuous. To solve this problem, a light layer of fusselerone was placed on the fabric but as it was a favourable medium for development of microorganisms there was contamination that reduced the efficiency of recovery of larvae.

3.2. Bubbling system

Finally as production increased, the handling of these containers was not cost effective and led to the assessment of the bubbling system by means of a pump for a fish bowl with a semi-industrial capacity. This method allowed us to handle a constant temperature within the bubbling bottle at 26-27°C and to assure hatching at the seeding time in 25% at 48 hours of incubation. It also made disinfection of the biological material feasible, avoiding an increase of the microbial population to the point of affecting eggs viability.

4. DIETS

Knipling [2], Guerra *et al.* [1] and other authors state that for mass rearing purposes, the insect must be reared at the lowest possible cost, which means that the maximum number of insects has to be produced with a minimum of material resources, manpower and time, without affecting the quality of the final product.

4.1. Gel diet

Originally, A. obliqua rearing was kept in a diet based on texturized beet but as it was not operative the search of a better alternative started. At the same time and to the end of increasing production, a diet of gel texture that follows the formula of the Welsaco, Texas, USA laboratories [5] was used. This diet is completely rich in nutrients for insects, although it has the inconvenience of a high cost and more handling during its preparation for mass rearing purposes.

4.2. Corn cob particle diet

In order to decrease costs and to keep the quality, a new diet was tested; it was based on corn cob particles as texturizer, sugar as a carbohydrate source, torula yeast as a protein source, and microbial inhibitors and vitamins as insect fortifier. Finally a standard formula was obtained with perspectives of increasing the egg to larvae transformation and assure a constant quality of the final product.

The only inconvenience of this formula is that contains hydrochloric acid which for rearing at massive levels presents a potential risk not only for personnel health but also for deterioration of facilities. For this reason, since the beginning of this year the gradual replacement of this ingredient has been under research. It has been replaced by citric acid which has shown promising results taking into account good production and quality and less risk in handling.

5. LARVAE

The percentage of larval recovery reflects the egg to larva efficiency in any mass rearing process. At the beginning there were problems to reach the present production levels, which were due to egg collection and poor larval diet. However, in mid 1996, larval recovery started to be more consistent as a result of changes made at the colony, mainly because of the establishment of the new oviposition panel and the use of the new larval diet; the colony slowly has been getting adjusted to mass rearing conditions. Figure 2 shows the larval increase obtained. At present an egg to larva conversion of 30% is obtained with a weight of 20 mg which favours 90% pupation at 24 hours.

Figure 2. Larvae production.

6. PUPAE

Since the beginning of the colonization the part of the process with less problems has been the larva to pupa conversion, which registers a transformation of 85-90%. However, it is important to mention that the optimum humidity range is 70-80% but if it gets out of this range the quality is seriously affected, and is reflected in the quality of the adult to be released in the field and in the colony members. These facts demand that environmental conditions remain at the established ranges.

Figure 3 shows monthly pupal production indices which have allowed maintenance of a constant number of cages in the colony. At present, average pupa weight oscillates from 15 to 16 mg.

Figure 3. Pupae production.

Special care has been given to the quality of *A. obliqua* since all the biological material is returned to the breeding colony. Liedo and Carey [3] recommend this special care in the rearing of flies that will form the colony to minimize adverse effects on quality of the produced flies.

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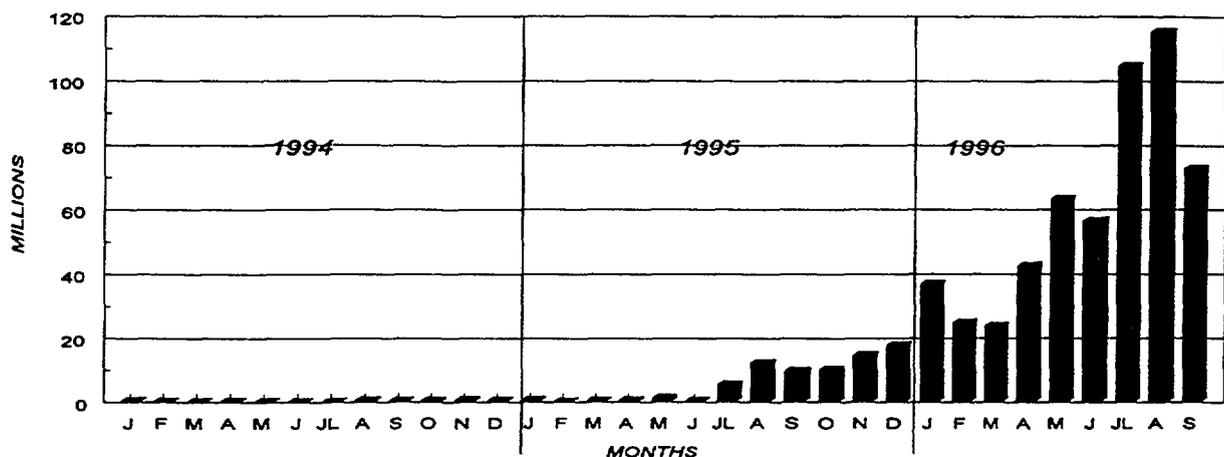
MASS REARING METHODS FOR FRUIT FLY

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Abstract

MASS REARING METHODS FOR FRUIT FLY.
The most common rearing methods used for mass rearing of fruit flies, with emphasis on those of economic importance in Mexico such as *Anastrepha ludens* (the Mexican fruit fly), *Anastrepha obliqua* (the mangoe and plum fruit fly) and the exotic fruit fly *Ceratitis capitata* (the Mediterranean fruit fly) are described here.

1. INTRODUCTION

The strategies for eradication of fruit fly utilizing the Sterile Insect Technique, have been adapted from the successful screwworm (*Cochylyomia hominivorax* Coquerel) eradication program, which has eliminated this pest from the southern United States, Mexico and a great part of Central America. This success, initiated in the 1960s, coincided with the elimination of the melon fly (*Bactrocera cucurbitae* (Coquillet)) from the island of Rot in 1962-1963 and the oriental fruit fly (*Bactrocera dorsalis* (Hendel)) from the island of Guam in 1963, (Fay, 1989).

Seven polyphagous species of Tephritidae have been identified for mass rearing or investigation: *Bactrocera tryoni* (Froggatt) (Queensland fruit fly), *Bactrocera dorsalis* (Hendel) (oriental fruit fly), *Bactrocera curcurbitae* (Coquillet) (melon fly), *Anastrepha suspensa* Loew (Caribbean fruit fly), *Anastrepha ludens* Loew (Mexican fruit fly), *Anastrepha obliqua* Macquart (West Indian fruit fly) and *Ceratitis capitata* (Wied) (Mediterranean fruit fly). All of these species are of tropical and subtropical origin and all are polyphagous and multivoltine, characteristics that have favored adaption to artificial rearing, in comparison to *Bactrocera oleae* (Gmelin) (olive fruit fly) or *Rhagoletis spp.*, which are monophagous or more host specific, which have made artificial rearing artificial more difficult for the moment, (Fay, 1989).

In Mexico we first began *Anastrepha ludens* rearing in the 1950s, when we obtained, in experimental form, small quantities of this species (SARH, 1995). The Sterile Insect Technique became very relevant in Mexico in 1977, when Mediterranean fruit fly invaded the south of the territory. Among the actions taken for integrated pest control was the release of millions of sterile flies, which were produced since 1979 at the rearing and sterilization facility at Metapa de Dominguez, Chiapas, Mexico, (Schwarz, 1985).

Mexico initiated an ambitious phytosanitary project with the National Fruit Fly Campaign against Mexican fruit flies, based on the success obtained from the eradication of Mediterranean fruit fly from south Mexico. Seeing the necessity of controlling the economically important *Anastrepha* complex, a fruit fly and parasitoid plant was built at Metapa de Dominguez, Chiapas, Mexico, which, along with the medfly production plant at the same place, forms the greatest bioindustrial complex in the world (SARH, 1995).

The present paper describes the most common rearing methods used for mass rearing production of fruit flies, with emphasis on those of economic importance in Mexico.

2. MAINTENANCE AND HANDLING OF THE COLONY (ADULTS)

2.1. Confinement of adults in cages

Adult fruit flies are confined in diverse cage designs, whose capacity depends on the amount of insect to be produced. These can be metal or board sheets with plastic screen ,which will allow correct ventilation and prevent insects from escaping (Vargas, 1989). Density of adults per cage can vary depending on the species and is the critical factor for survival and fertility. Density effects should be studied in order to determine the size of the cage and the population level confined (Fay, 1989). In the case of *Anastrepha ludens*, from Mission, Tex., we have a report of 110,000 flies per cage and 140,000 in Metapa, Chiapas, Mexico. In *Anastrepha obliqua* the density handled per cage is 50,000 flies. In both cases the cage is 1.5 m high X 1.4 m wide X 0.3 m deep, so these species use similar sizes. Among the smallest tephritid is the Mediterranean fruit fly (*C. capitata*), which confines densities of 300,000 adults in a cage 2.8 m high X 2.0 m wide X 0.2 m deep.

2.2. Oviposition site

The most common way of collecting *C. capitata* eggs is by using screens or cloths, where the flies lay eggs, which are then collected in water containers, or the use of opened bottles which are removed after the collection, as done in Hawai. In the case of Mexico, Guatemala, Chile and Argentina, *Ceratitis capitata* lay eggs through a screen covering the cages and these fall into the water collectors. In *Anastrepha* spp., the ovipositor is thicker and needs a harder substrate, which allows major mechanical stimulation. For this reason screens with different hole sizes, smaller than those for *C. capitata*, are used. A peculiar characteristic of the oviposition substrate for *A.ludens, A. suspensa* and *A. obliqua*, is that these are covered with silicon or cement screen (for *A. suspensa*). This is a favorable characteristic because, for these species, egg collection is done by water spraying, allowing the eggs to fall towards the back of the oviposition chamber and inside the container. The tape placed on the oviposition substrate avoids humidity to be introduced inside the cage which would cause contamination.

The oviposition sustrate or panel is placed on the lateral area of the cage and occupies one third of the lateral space, from which the oviposition chamber overviews. Here the panel is separated from direct contact from the environment by means of a door, normally acrylic, in order to view the interior of the cage without trouble.

In these species of *Anastrepha*, dehydration of the egg is carefully avoided, separating it from the oviposition chamber and covering it twice daily with fine gel (furcelleran) to maintain the egg moisture until collection the following day. A variant for *A. suspensa* is the use of sponges placed all along the ovipostition chamber door, this causes a damp environment within the chamber, maintaining egg hydration. This problem is solved in *C. capitata* from the moment the egg is laid and drops into the water container, from where is is then collected.

In order to induce artificial oviposition in rearing of flies for experimental levels, a dome simulating a host fruit is used. This dome is covered with cloth with a hot parafin screen, which becoming solid and forms a soft surface, simulating the host fruit. Here the fly lays its eggs, which are then collected with water inside the cavity formed within the dome.

2.3. Sexual maturation of adults

The period of oviposition for *Anastrepha* species fluctuates from 8 to 10 days, while for *C.capitata* it is from 2 - 3 days.

The copulation period for mature adults occurs from 1-2 days before the preoviposition period. This point is very important when considering plans for how long flies will remain in the cage for productivity. This goes hand in hand with the longevity of cage-reared adults, which varies according to the species, the density of adults per cage, atmospheric conditions and with food and water.

2.4. Fertility

The fertility of a species can vary depending on the results obtained from the medium used, such as artificial oviposition substrate In the typical case, exposing female flies to preferred hosts will greatly increase fertility. This is why the selection of the artificial oviposition substrate in mass rearing of insects should be based on the natural wild behavior of the insect. Copying the natural atmospheric condition for artificial rearing will also help the insect gradually adapt.

Schwarz et al. (1985) report approximately 160 eggs/female *C. capitata* during a period from 12-13 days under lab conditions. Mission, Texas, rearing of *A. ludens* reports 560 eggs/female in 20 days of productive life, and, in the same species at Metapa, Mexico, there is average production of 464 eggs/female in a productive period of 13 days. In the case of *A. obliqua* we have values of 108 eggs/female in a period of 13 days at Metapa, Mexico, and 300 eggs/female in Weslaco, Texas. With the latter, we utilize domes with parafin and in the other cases with *Anastrepha* we utilize cloth panels covered with silicon.

By the experiencies obtained, it is not recommended to maintain adults confined for more than three weeks, because during this period great mortality leads to low egg production. Egg quality also starts decreasing during this time.

2.5. Food and water for adults

The traditional food for tephritids is sugar, enzymatically hydrolyzed protein and water, which provides the necessary amount of protein for egg development and maturation. Other sources explored are honey and fruit parts.

For *Bactrocera oleae* (Tzanakakis, 1992) liquid and solid food have been evaluated, where the mixture 1/4/5 of enzymatically hydrolyzed yeast, sugar and water, respectively, provides high fertility in females. Liquid food is rapidly consumed and solid food is consumed more slowly, with positive results with liquid diet and higher amount of egg production. The disavantage of a liquid diet is that it needs to be replaced two times a week, due to its rapid dehydration. For solid diet the advantage is that it lasts throughout the egg production period, although the area is damp, tends to become sticky, and flies are caught.

Actually, solid food is used most, varying with the addition of casein, cholesterol, vitamins, which may affect fertility and fecundity. Water is obtained by means of cotton, napkins, sponges or damp filter paper in contact with water contained it PVC tubes or their

equivalents. Water is generally chloride at a minimum of 2 ppm or treated with ultraviolet light to reduce microbiological contamination risks in adults.

2.6. Atmospheric conditions of the colony

Species of the tephritid family are mainly tropical or subtropical and need hot, damp conditions for optimum egg production. The range of temperature and humidity considered ideal fluctuates from 25-27°C and 50-80%, respectively, (Fay, 1989).

Another factor regulated in the colony is light, which in most of the cases is on continuously. This increases egg production compared with intervals of light/darkness. For *Anastrepha* spp. and *C. capitata*, light has been intensified from 3,000 to 10,000 lux (Fay, 1989). For *A. ludens*, *A. obliqua* and *C.capitata* in Metapa, Mexico, periods of light/darkness are utilized with excellent results on egg quality (percentage), maintaining adequate egg production.

It is important to note that during copulation, tephritids prefer crepuscular light, be it morning, afternoon or both. For these periods of light/darkness should be considered at least 12:12 hrs.

3. EGG HANDLING

3.1. Collection

For *A. ludens* and *A. obliqua*, eggs are collected daily from the oviposition panel with a fan spray which sprays water finely and eggs fall directly on the container. For *C. capitata* the egg is laid through a fine mesh and falls in a water collector, from where it will then be removed (Schwarz et al. 1985).

After collection, eggs are submitted to a disinfection treatment using, preferably, chloride at 250 ppm and sodium benzoate at 0.07%, for *A. ludens* in Metapa, Mex. For other species, such as *A. obliqua* and *C.capitata*, eggs should be washed only with distilled water and treated with ultraviolet light; no disinfectant should be added.

Fay (1989) reports the use of hydrochloric acid at 0.025% for 20-30 seconds for egg disinfection, washing them afterwards with clean water or using sodium benzoate at 0.03% to disinfect the egg's surface from microorganisms.

3.2. Incubation

Prior to collection, eggs continue in the incubation phase, under controlled temperature conditions, mainly fluctuating from 26-28°C. Under these conditions, *A. ludens* takes 4 days for incubation, *A. obliqua* 3 days, and *C. capitata* 2 days. The traditional incubation method is to use paper napkins or strips of filter paper, here the eggs, covered with hydrating gel, are added. These can be contained in petri dishes or other containers of greater dimensions hermetically sealed and with rigorous temperature control in incubation equipment. A variant is the use of water incubation, contained in bottles, in the ratio of 1:20 water:egg, oxygenated by air injection coming from specialized equipment, with air purifying filters.

This method is easier to implement when large quantities of eggs are handled and required handling space is less, with excellent results for *C. capitata, A. ludens, A. obliqua,* which present probability percentage above 90%.

3.3. Egg seeding

When egg eclosion reaches 5-25%, or more, in the water incubation system (up to 60%), eggs are ready to be seeded on larval diet.

Eggs will be seeded depending on the incubation systems. In the case of incubation on towels or strips of paper, the number of strips to be placed on the trays will be designated, depending on the density of egg/gram of diet. For *A. ludens* we handle 7.5, while for *A. obliqua* the report is of 7.75 and 14 for *C. capitata.* These are the values handled at Metapa de Dominguez, Mex., for mass rearing levels of the respective species.

4. ARTIFICIAL LARVAL DIET

4.1. Ingredients

One of the major efforts that has been invested in improving rearing of tephritids has been the larval diet. A diversity of ingredients has been used for rearing different species of tephritids, most of which have the same characteristics in common (Fay, 1989).

The major component is water, and its quantity depends on other components used, the physical characteristics and environmental conditions to which the diet is exposed. Water plays an important role in minimizing metabolic heat generated during the last larval stages, besides being the vehicle by which larvae obtain their nutrients.

The ingredients that occupy the second place in larval diet proportion, are texturized or volume agents, the most important of which are carrot powder, corn-cob powder, texturized soybean, wheat bran, wheat buds, wheat grits, cane bagasse, beet pulp (sugar beet), ground pasture, ground paper, corn flour, coffee husks, etc. All of these ingredients have in common a high fiber content (cellulose), which permits an optimum medium for larval development. The convenient amount of these ingredients depends on the species of tephritid to be reared, which oscillates between 5 and 26% of the total diet formula. Some of these ingredients provide nutrients to the larvae, including carrot powder, which is a source of vitamin A and carotenes, and grains from wheat by-products, which contain more or lesser protein and sterols grade. Hence, the use of these two ingredients is widely defended (Fay, 1989).

Protein sources explored have been by excellency beer yeast (*Saccharomyces cereviseae*) and torula yeast (*Candida utilis*), which, beside containing from 45 to 50% protein, are rich in vitamin B (B complex), carbohydrates and minerals. Other sources of proteins utilized are casein, soy flour, cotton seed and wheat sprouts, utilized in a lesser degree as a complement for the yeasts. The principal function of proteins is for construction and regeneration of cells and organs (Fay, 1989).

Carbohydrates are commonly obtained from granulated sugar, which has a stimualatory effect on the insect. Other carbohydrate sources are texturized, such as soyflour, sugar cane bagasse, wheat by-products, rice bran and corn flour among others, besides yeast.

Carbohydrates contribute to the function and structure of insect tissue and can be found in the nucleus, cytoplasm and cellular membrane, as well as extracellular hemolymph as support for the tissue, (Fay, 1989). The principal input of fats (lipids) is constituted by dried inactive yeasts, as well as in some cases some textures such as soyflour, corn flour, wheat by-products (principally wheat sprouts). Lipids are essential components of cellular membranes and act as precursor of ecdysone hormone (principally sterols), essential constituents of epidermis, forming an impermeable screen which stops insect dehydration, and for prolonged flight (Dadd, 1973). In the case of vitamins, ascorbic acid (Vitamin C) is present in most of the diets for phytophagous species and is reported as constituent of tissues preventing infectious diseases.

Vitamin A is associated with the normal vision of the insects. The function of Vitamin E is to protect the cellular membranes, reducing toxic by-product of peroxidation of polyunsaturated fatty acids. Vitamin E is also associated with egg production and sperm probability (Sivrastava et al. 1977).

The B complex is reported as essential in the insect diet and is supplied by adding dried inactive yeasts. Insects also require substantial quantities of minerals such as potassium, phosphorous and magnesium, and small quantities of calcium, chloride phosphate and iron outlines, zinc, magnesium and copper, which act as enzymatic cofactors for reactions that occur in insect metabolism (Schwarz, 1989).

Most of the textures are agricultural by-products and have no rigorous quality control. Hence, by nature, they have a high microbial count (bacteria, fungi and yeasts). When used with other diet ingredients, microorganisms spontanously reproduce in this appropiate medium; for this reason, we commonly resort to the use of microbiological inhibators and acidulating agents to avoid food contamination and harm to the insect. Methyl paraben (Nipagin) is used for controling yeasts and fungi, and to a lesser extent for bacterias (especially gram-negative), in pH ranges of 4 to 8. Sodium benzoate is effective against yeasts and bacteria and, to a lesser extent, against fungi, at ranges of pH of 2.5 to 4.0, (Baudi, 1989).

Other microbiological inhibitors utilized are propionate and sorbate by-products. Cholidric and citric acids are normally used to obtain the desired pH, which are adequated acccording to optimum pH for antimicrobial action of microbiological inhibitors and optimum pH for larvae development (commonly between 3.5 and 5.5.), (Funke, 1983). Chart No. 1 presents different formulae for larval diets, for the mass rearing of different tephritid species.

4.2. Preparation of diet

Diet is prepared with special mixers designed according to the maximum amount of daily preparation and components of diet. If these are of particle or fiber size, its preferable to use a pallet mixer, since it is easier to homogenize and mix in less time. When the size of the particle is smaller, such as flour, stripped mixers are recommended, because of its configuration homogenizes small particles at maximum. Normally ingredients are mixed dry at the beginning (texturized, yeast, sugar, etc.), forming a homogenized mixture, and then water with previously diluted preservatives is added.

Chart No. 1. COMPARATIVE CHART OF DIFFERENT LARVAL DIET FORMULAS FOR MASS REARING OF DIFFERENT TEPHRITIDS

Ingredients	C. capitata (Mexico)	C. capitata (Guatemala)	A. ludens (Mexico)	A. ludens (USA)	A.obliqua (Mexico)	A.obliqua (USA)	A.suspensa (USA)
Corn-cub powder	--------	-------	17.2	15.82	15.47	--------	26.44
Wheat bran	16.1	16.0	----------	---------	----------	---------	-----------
Soyflour	16.1	----------	----------	---------	----------	---------	----------
Corn flour	----------	----------	5.3	---------	8.25	6.0	----------
Sugar cane Bagasse	----------	9.9	----------	---------	----------	---------	----------
Wheat bud	----------	----------	----------	8.43	----------	---------	3.12
Dried Yeast	8.6	9.9	7.0	6.32	6.01	4.52	4.15
Sugar	8.3	9.9	9.2	7.59	8.59	8.0	6.23
CholidricA.	----------	0.44	----------	0.95	0.56	0.5	0.73
Citric acid	0.7	----------	0.55	---------	----------	---------	----------
Sodium B.	0.25	0.29	0.40	0.10	0.24	0.1	0.11
Methyl Par.	0.65	----------	0.20	0.23	0.12	0.1	0.21
Guar gum	----------	----------	0.10	---------	0.1	---------	----------
Vitamins	----------	----------	----------	0.23	0.4	0.4	----------
Casein	----------	----------	----------	---------	----------	2.33	----------
Cholesterol	----------	----------	----------	---------	----------	0.05	----------
Beck salt	----------	----------	----------	---------	----------	0.08	----------
Agar	----------	----------	----------	---------	----------	0.3	----------
Water	49.3	54.0	60.05	60.45	60.20	77.62	59.01

Time of mixture depends on the nature of ingredients, thicker particles require less time for homogenization, while those with thin particles need a little more time. These values should be determined according to the final texture desired for the diet, taking care that the time of mixture is not excessive to avoid overheating of the diet, which could cause the quality of the nutrients to be lost.

5. LARVAL DEVELOPMENT

5.1. Containers

Larvae can be fed in a large variety of containers (trays, boxes, etc.). The model and configuration of the container will be determined by the amount of diet used and the volume it represents. The container should offer the facility for wide disipation of metabolic heat generated by the larvae, should be durable, easy to wash and if more space is required then it should be handy. Containers or trays could be aluminum, plastic or fiberglass, and are commonly rectangular.

5.2. Distribution of diet inside tray

Prepared diet is mesured by weight into the tray, and distributed homogeneously throughout the container, assuring a flat surface so that it is ready for egg seeding; eggs mst be distributed uniformly on the surface. The amount of diet per tray varies from 5 to 9 kg depending on the size of the tray.

5.3. Larval stages

Tephritid larvae pass through 3 larval stages within 5-9 days before completely maturing. In Metapa, Mexico, *A. ludens* completes its development in 9 days, *A. obliqua* in 8 days, and *C. capitata* in 7 days. *A. ludens* in Mission, Texas, USA, under different diet conditions, completes its development in 8 days, *A. suspensa* in Gainesville, Florida, USA, completes its development in 9-10 days.

In some cases, larval development requires two or three different rooms, in order to control temperature during each development stage. Temperatures for larval development range from 25°C to 28°C and relative humidity from 60-85%. Normally, to synchronize egg eclosion and homogenize the age of the larvae, its recomended that for the first stage the temperature should be 28-30°C, reduced to 27-28°C for the second stage and 25-27°C for the third. Table No. 2 presents various cases of atmospheric and handling conditions for different tephritid species.

5.4. Survival

Percentages of larvae-egg recovery are increased when a major adaptability of the insect in larval diet exists and when the production control process is standardized. The optimal level of larvae-egg recovery is estimated at 50%, however, some offspring, such as *C. capitata*, are reported at 60-70%.

Variation in larval production depends to a large extent on the quality of ingredients and fluctuations in atmospheric condition (out of parameters) or a combination of both factors. Under this situation, the rigorous control of ingredients is imperative, according to parameters determined as ideal for each species of insect.

5.5. Larval separation

The most common methods of separating larvae are: larvae jump (self-popping), sifting tumblers and damp separation. The method varies according to the species; hence, the popping method is utilized for *C. capitata, B dorsalis, B cucurbitae and A. suspensa*. With this method, larvae abandon the tray freely upon completing the third instar , jumping from the tray and dropping into water or another pupation substrate (vermiculite, sand, sawdust). With the tumbling method, diet is removed to break compaction and then dumped inside a mechanical sieve, which turns around intermitently, allowing larvae to be separated from the diet (Vargas, 1989). This method is used for *C. capitata* and *A. ludens* in Mexico and the United States.

The third method is damp separation, where the food is diluted in a water container, and injected with air pressure to maintain the water-diet-larva mixture in a homogenized form.

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Larvae are then extracted from the container by means of PVC tubes connected to the base of the container, and joined by another Y tube connection, where air is generated (ventri effect) while injecting water at high pression, which pulls the water from the container and into manual sieves, where larvae are caught and diet is washed out. This method is utilized for *A. obliqua* and *A. ludens*, in circumstances where the diet is too damp and compacted to permit separations with the other methods.

6. PUPAE

6.1. Pupation methods

Once larvae are collected, they proceed to pupate on the pupation substrate, which could be vermiculate, sawdust, sand, inclusive wheat (wheat, rice).

The pupation sustrate simulates the ground where larvae normally pupate after leaving the fruit and is widely used for *Anastrepha* species and for *C. capitata*. A variant is naked pupation, used in Mexico for *C. capitata*, with excellent results (Shwarz et al. 1985), where extreme care should be taken to maintain high humidity to avoid loss of pupal weight.

6.2. Atmospheric conditions

In the tephritid species that are mass reared, a room for pupation is usually utilized where temperature is lower than the room assigned for pupal maturation. The objective is to counteract metabolic heat caused by larvae inside the container (tray). This room is also characteristically dark to minimize the number of larvae that pop out of the tray. Temperature for pupal development normally ranges between 24 and 20°C.

Another important factor is relative humidity, which should remain between 70 and 80% to avoid pupal weight loss. Generally, moisture (10-20 %) is added to the pupation substrate to help pupae retain weight.

Chart 2 presents values of temperature and relative humidity utilized for some tephritid species during the development phase for artificially reared pupae. Under these parameters, pupal deveopment is completed in 13 to 14 days for *A. ludens* and *A. obliqua* in Metapa, Mexico, Mission and Weslaco, Texas, USA, respectively; 12 days for *C. capitata* en Mexico, and 11-15 days for *A. suspensa* in Gainesville, Florida, USA.

6.3. Survival

Recovery of larvae to pupae varies between 80 and 90% in tephritid species, depending on adequate nutrition of the larvae and good control of atmospheric conditions. These factors are also closely related to the transformation percentages from pupae-adults, for which values of 75-95% are considered acceptable.

6.4. Separation and dyeing of pupae

To separate pupae from substrates (vermiculite, sawdust, etc.) a sieve is commonly used, which can be mechanical or manual (tumbler). This eliminates substrates through the screen and clean pupae are retained. This method, although the most common, is reported to

Chart No. 2. TEMPERATURES AND RELATIVE HUMIDITY FOR DEVELOPMENT STAGES OF FRUIT FLIES, FOR MASS REARING OF DIFFERENT SPECIES OF TEPHRITIDAE.

Area	C. capitata (Mexico)		A. Obliqua (Mexico)		A. ludens (Mexico)		A. ludens (USA)		A. suspensa (USA)	
	°C	R.H.	°C	R.H.	°C	R.H	°C	R.H.	°C	R.H.
Colony	27±1	70±5	27±1	75±5	26±1	55±5	24±1	65±5	26-27	65±5
Incubation	26±1	-----	26±1	------	26±1	------	25.5	------	24	-----
Larvae I (1st. Stage)	30±1	90±5	28±1	90±5	28±1	85±5	28±1	80±5	27-28	80±5
Larvae II (2nd. Stage)	28±1	70±5	27±1	70±5	27±1	75±5	25.5±1	70±5	27-28	80±5
Larvae III (3rd. Stage)	28±1	70±5	26±1	70±5	26±1	75±5	25.5±1	70±5	21-22	90±5
Pupation	23±1	70±5	20±1	70±5	21±1	70±5	24±1	70±5	25.6-26.6	70±5
Pupae maturation	25±1	70±5	24-25	80±5	25±1	75±5	26±0.5	70±5	25.6-26.6	70±5
Packagin Room (Chill Room)	15-18	70±5	20±1	75±5	19±1	75±5	15.5	70±5	15.5	70±5

harm the wing muscles of some tephritid species (Osaki and Kobayashi, 1989), at level of up to 33% in *C. capitata, B. dorsalis,* and *B. curcurbitae.*

To solve this problem, in Metapa, Mexico, a wind tunnel is utilized, powered by a fan in a closed structure (tunnel form) covered in its interior with foam rubber to temper pupae while falling from the dosificator until they are in contact with air at moderate speed. This air speed is sufficient to carry vermiculate out by difference in weight, and pupae fall on the container, free of substrates. This method is utilized for *A. ludens, A. obliqua* and *C. capitata,* with highly satisfactory results.

After separating the pupae, a certain percentage is returned for maintaining the colony and the rest is designated for release following irradiation. Pupae utilized for autocidal control are marked (dyed) with a phosphorescent dye (Day-glo), which allows diffirentiating sterile from wild adults when they are captured in trapping programs. The amount of the dye normally utilized is 1-2 grs. per liter of pupae. Dyeing of pupae is done in hermetically sealed plastic bottles or inside plastic bags. A designated volume of pupae corresponding to the amount of dye is placed inside, and these are slowly mixed with an oscillating movement until dyeing of the biological material is obtained.

7. PACKAGING AND IRRADIATION OF PUPAE

7.1. Selection of pupae

Irradiation of pupae normally occurs 2 days before adult emergence. The physiological age of a pupa is measured by determining the imago coloration (eye coloration),which varies

according to pupal age and is a simple parameter to use to determine an appropriate moment for irradiating pupae.

Color varies from a creamy white in young pupae to iridescent green in mature pupae of *A. ludens, A. obliqua* and *A. suspensa* and ochre red in *C. capitata*.

7.2. Hypoxia

In order for the action of irradiation to be efficient for inducing sterility of adult tephritids, pupae are exposed to a hypoxia process from 1-2 hours before irradiation and it is recommended that they be kept in this condition at a temperature of 15-17°C, so that under these circumstances, physiological activity (cellular division) of the insect is reduced to a minimum and direct harm to the cells of the reproduction organs can occur, which continue to divide.

7.3. Packaging

The hypoxia process normally occurs inthe packaging (Sausage bags or plastic pans) in which pupae are deposited to be transported to reception centers. If biological material is transported long distances, they should be transported in specially designed boxes for correct handling. In most cases, blue ice is utilized to maintain interior temperature in the box at 25-27°C.

7.4. Irradiation dose

Ability to induce high levels of sterility with minimum deterioration of insect quality is a basic requirement for any program utilizing the SIT (Hooper, 1989).

When reproductive cells are exposed to ionizing radiation, lethal dominant mutations result. In the Diptera, these mutations consist of loss of formation of chromosome fragments and/or formation of acentric and decentric chromosomes, which do not allow the adequate balance of chromosomes in the zygote. Thus, they die early in development (Hooper, 1989).

In Metapa, Mexico, the radiation dose for *C. capitata* is 14 krads (^{60}Co), to obtain 90% sterility, or inclusive higher. For *A. ludens* and *A. obliqua* 8 rads (^{60}Co) is utilized to obtain a similar sterility level.

In Mission, TX., USA, the same dose and same sources are used for *A. suspensa*. A radiation dose can vary depending on the presicion of the radiation source and age of pupae. As noted by Hooper (1989), sterility of 95-100% affects competivity of *C. capitata* males. The dose should be reviewed depending on the geographic location of the fly due to possible variation on genotypic characteristics.

8. HEALTH

As a preventive measurement in any installation for mass rearing, aseptic measures should be implemented to minimize contamination risks by microorganisms pathogenic for the insect.

Cleaning should be focused on material and equipment utilized for the different phases of insect development, as well as on the building. Use of sodium hypochlorite at 5-6% is recommended, and for cleaning activities for places with contamination risks, ammonium quaternary salts at 1% should be used.

9. FUNDAMENTAL ASPECTS FOR TEPHRITID MASS REARING

The greatest effort has focused on reducing operating costs on the most significant point, which is larval diet. Larval diet represents 40-50% of the investment for operation of the mass rearing plant. Alternative sources of ingredients of lesser price are a resource frequently used and can be combined with optimization of physical and chemical characteristics of the diet for better development of larvae.

Variation in insect quality is frequently caused by the quality of larval diet ingredients. These should be adjusted within the most strict parameters for physical-chemical, microbiological and nutrition, with the goal of obtaining the most standard ingredient.

The most important goals in application of SIT are to produce and release enough adults of optimum quality to cope with wild adults and be able to suppress or eradicate fly populations. To maintain a constant good quality and assure competitiveness of sterile adults in the field, the permanent procedures for rearing should be improved for better management in the different stages of insect development, adapting biology and behavior of the insect to the artificial rearing system.

Finally, the parameters for evaluating insect quality should be designed to predict the moment when it is necessary to replace the laboratory colony with wild flies, taking care to maintain the sexual aggressiveness of adults in order to achieve the principal goal of controlling and suppressing wild flies.

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CURRENT INITIATIVES IN THE MASS PRODUCTION AND FIELD RELEASE OF THE MEXICAN FRUIT FLY, *Anastrepha ludens*, IN THE LOWER RIO GRANDE VALLEY OF TEXAS

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Abstract

CURRENT INITIATIVES IN THE MASS PRODUCTION AND FIELD RELEASE OF THE MEXICAN FRUIT FLY, *Anastrepha ludens*, IN THE LOWER RIO GRANDE VALLEY OF TEXAS.

In order to reduce program operating expenses in the South Texas Mexican Fruit Fly Sterile Release Program, four cost reduction initiatives are in progress at the U.S. Department of Agriculture's Mexican Fruit Fly Rearing Facility. These initiatives include implementation of a less expensive larval diet formulation, automation of the larval diet dispensing process, processing and reutilization of spent larval diet medium, and a more efficient system for emerging and feeding sterile flies prior to field release.

1. INTRODUCTION

The Mexican Fruit Fly, *Anastrepha ludens*, Loew, a destructive pest of citrus was first reported infesting fruit in South Texas in 1927. Since the Mexican fruit fly was not established in other citrus producing areas of the U.S., all fruit originating in South Texas for shipment to these areas required treatment with the fumigant ethylene dibromide (EDB). In 1984, the U.S. Environmental Protection Agency, banned the use of EDB on citrus destined for consumption in the U.S. From 1984 to the present time, the sterile release program has been the principal means of fruit certification in the Rio Grande Valley of Texas. Funding for the program is provided by The Citrus Industry, The Texas Department of Agriculture and The U.S. Department of Agriculture. In order to maintain program viability within the constraints of limited funding it has been necessary to reduce program expense wherever possible. Four such cost reduction initiatives are discussed here.

2. DESCRIPTION OF INITIATIVES

2.1. Development of less expensive larval diet formulations

The larval rearing medium for the Mexican fruit fly was adapted from the fresh carrot medium developed in Hawaii by substituted powdered dehydrated carrot for fresh carrot but results were inconsistent when used in mass production. Rhode and Spishakoff [4], found that the addition of dried torula yeast, a non-fermenting, dietary supplement, produced more consistent results and improved yield and larval weight. The torula yeast-fortified, dehydrated carrot diet medium was the standard used for mass production of the Mexican fruit fly from 1965 until recently.

The quality and cost of both dehydrated carrot powder and torula yeast have proven to be highly variable and problems with quality have produced disastrous production results on numerous occasions. Additionally, carrot powder is perishable and storage at room temperature is limited to approximately three months. For these reason, a search for alternative ingredients was undertaken.

2.1.1. Replacement of dehydrated carrot powder

The quality of dehydrated carrot powder was highly inconsistent and production results were variable. For this reason, a replacement for carrot powder was the first priority. A wide variety of dried, powdered materials were tested but wheat germ was found to produce superior results (Table 1). While raw wheat germ produced slightly better results, stabilized wheat germ, or wheat germ that has been heat treated to reduce enzymatic activity, is the ingredient of choice due to its extended shelf life.

2.1.2. Replacement of torula yeast

Torula yeast was chosen for replacement primarily due to its high cost. Earlier tests had shown that torula yeast could be reduced by 50% (6.4% to 3.2%) and replaced with raw wheat germ and a vitamin supplement and meet all of the quality control parameters. The complete removal of yeast from the larval diet and replacement with raw wheat germ increased the larval development period by two days and decreased yield by approximately 20 percent. The quality control parameters were normal. Jang [5] demonstrated the importance of niacin, calcium pastothenote and riboflavin in the nutrition of medfly. Larval yield and ratio of development were normal when these B vitamins and Roche vitamins mixture were added to the diet. Production and quality control parameters comparisons of the standard torula yeast and yeast free diet are found on Table 2. The comparison of the carrot powder, wheat germ and no yeast larval diet medium, expressed as percentage by weight, is found in Table 3.

2.2. Automation of larval diet dispensing process

The larval diet medium used at the USDA facility in Texas consists of dry, finely ground nutritive ingredients, preservatives, acid and water (Table 3). The consistency of the finished product is semisolid and conveyance of the material from the mixer to the tray loading area has typically been performed through the use of a screw type auger. While the auger is an adequate means of conveyance it is not useful for accurate dispensation of product. Further, when dispensing a semisolid material such as the larval diet medium, it is necessary to manually spread the material and smooth the surface prior to the seeding of eggs. This process was very labor intensive and was identified as an area where cost savings could be obtained through automation.

Engineers at the USDA, APHIS, PPQ Aircraft and Equipment Operations Center, Edinburg, TX began investigating commercially available pumps that were capable of conveying the diet medium with enough force to extrude the material into a smooth surfaced sheet while being dispensed into the diet tray. Several designs showed promise but the most compact, least expensive design was the sinusoidal rotor pump, model MR-135-RF manufactured by the Sine Pump Company, Orange Mass. 01364. A pneumatic motor, axial piston design, model number 73337AA7, Cooper Industries, Gardner-Denver Division, Lexington, S.C. 29072, was chosen as the pump drive motor due to the repeated cycling

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Table 1: CARROT POWDER REPLACEMENT

Ingredient	Pupal Wt Mg	Pupal/ Kg of diet	% Eclosion	% Fliers
Carrot Powder	7.4 ± 1.5	5,458 ± 532	96.7 ± 4.4	94.7 ± 4.0
Stabilized W.G.	20.2 ± 1.6	4,993 ± 846	96.7 ± 1.5	93.7 ± 3.1
Corn Germ Cake	18.9 ± 1.4	4,393 ± 1039	95.0 ± 2.4	91.2 ± 2.7

Means ± SD

Table 2: TORULA YEAST REPLACEMENT

Diet	Pupal Weight (mg)	Pupae Per kg Diet	Percent Emergence	Percent Fliers
Torula Yeast	20.7 ± 0.75	5,522 ± 451	98.4 ± 0.75	95.5 ± 2.5
Yeast Free	18.8 ± 1.31	5,805 ± 698	98.4 ± 1.1	94.9 ± 5.4

Means ± SD

Table 3: MEXICAN FRUIT FLY LARVAL DIETS. MISSION MEXICAN FRUIT FLY FACILITY; MISSION, TEXAS

Ingredients	Carrot Powder	Wheat Germ	No Yeast
Water	66.8	59.6	60.00
HCL	0.66	0.95	0.95
Sodium Benzonate	0.08	0.1	0.1
Methyl Paraben	0.21	0.23	0.23
Corn Cob Fractions	11.93	15.8	15.8
Sugar	7.42	8.40	8.4
Carrot Powder	7.42	--	--
Torula Yeast	5.44	6.3	--
Wheat Germ	--	8.4	14.86
Guar Gum	--	0.1	0.1
Vitamin Mix*	--	0.1	0.1
B Vitamin Supplement**	--	--	0.0025

*Vitamin Premix, Roche Vitamins and Fine chemicals, Hoffman-LaRoche, Inc., Nutley, New Jersey 07110.
**D-Calcium Pantothenic acid, Riboflavin, and Niacin @10:5:10 mg/Kg of diet.

required for dispensing into individual trays. When in operation, the pump is controlled by a programmable digital revolution counter which controls a pneumatic solenoid valve on the air motor. Magnetic sensors on the pump shaft send pulses to the revolution counter and when the programmed set point is reached power to the solenoid valve is interrupted.

The labor required to dispense diet and infest trays was reduced by more than 50 percent by implementing the diet pump.

2.3. Processing and reutilization of spent larval diet medium

The majority of mature Mexican fruit fly larvae will pupate in the spent larval diet medium if not removed. The standard procedure used for Mexican fruit fly larval collection has been to dilute the spent diet medium containing larvae (and some pupae) with water, agitate the slurry with compressed air and collect the larvae by passing the slurry through a screen sieve. This system results in the loss of the spent diet medium and the creation of much waste water. Additionally, the larvae collected from the water must be placed in a pupation substrate, such as vermiculite or sawdust, to reduce mortality during pupation.

Tests are in progress to replace the water sieve larval collection process with a dry method utilizing a rotary screen sieve. The spent diet medium is dried thoroughly during the last 24 hours of larval development by using dehumidifiers and wall mounted fans. The dried, granular spent medium is then poured into the hopper of the rotary screen apparatus. The screen cylinder, 61 cm in diameter x 175 cm in length, rotates at 4.6 revolutions per minute. The cylinder has two screened sections, the first, measuring 91.5 cm in length consists of screen with hole opening size of 2.4 mm. The second screen is 30.5 cm in length with an opening size of 6 mm x 18 mm. The dried diet medium passes through the first screen into a collection cart situated beneath the screened cylinder. The larvae continue down the inclined screen cylinder to the second screen where they exit and are collected in plastic pails. The surface of the diet medium often forms a crust that is difficult to break up and exit with the granular material. The large pieces (> 6 mm in diameter) exit the end of the cylinder and are collected in plastic pails. This material is combined with the granular material and processed for use as animal feed, soil conditioner or other purposes.

Efforts are currently underway to process the spent diet medium by pelletizing. The pelletizing machine requires that the diet moisture content range between 8-15 percent.

The pelletizer will destroy residual Mex fly larvae and produce a product which can be stored in reduced space for extended periods. The new larval collection and diet disposal system will hopefully be operational in the near future.

2.4. Emergence and feeding of sterile flies prior to field release

Sterile fruit flies are typically released by air as either active or chilled adults. Sterile flies used in the South Texas SIT program are emerged from pupae in screen panelled, plastic boxes measuring approximately 50 cm x 60 cm x 30 cm. Each box holds 6 paper bags containing 4,000 pupae each. The bags are loosely closed with staples to permit exit of the emerging flies from the bag into the box. Food in the form of 1% agar, 24% corn syrup and 75% water is placed on a screen panel centered on the lid of the box. Flies obtain water and nutrients from the food.

These emergence containers are commonly called PARC boxes (Plastic Adult Rearing Container) and are an improvement over earlier containers made of cardboard. They are durable and can be cleaned with water after each use. However, there exists inefficiencies with this system such as poor space utilization, high expendable supply cost and excessive insect escape.

A new system is presently being investigated which, if successful will either eliminate or reduce the extent of the aforementioned problems associated with the PARC box. The basic unit consists of an aluminum window screen frame measuring 76 cm x 76 cm constructed of 2.5 cm x 2.5 cm aluminum square tubing. Each frame is fitted with polyethylene screen material with a opening size of approximately 1.2 mm. A diagonal brace, located on each inside orner of the frame, forms the pupae loading area of the system. The diagonal brace is constructed of perforated aluminum with perforation hole size of 2.4 mm. A cloth panel, 15 cm x 15 cm, is sewn into the center of the screen panel and serves as a feeding site.

In operation, approximately 3,250 irradiated dyed pupae are poured into each of the four pupae compartments. The individual trays are stacked one on top of the other to a height of 185 cm, which is a total of 73 trays. The top tray forms the lid and is not loaded with pupae. The adult flies emerge from the pupae for a period of 5 days. The emerging adults escape the pupae compartment by crawling over the top of the perforated aluminum partition, or passing through the perforations. Once the flies are free of the pupal compartment and within the confines of the stacked trays, they obtain water and nutrition from the cloth pad located on the center of each tray. A mixture of 5% sucrose, 0.02% sodium hypochlorite and water is fed the flies by using a drip emitter tube located at the top of the tray stack. The liquid is dispensed at a constant rate of approximately 400 mls per hour throughout the 5 day eclosion period. Some further refinement of the feeding system is required at this time. This work is in progress.

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ENSAYOS PARA DETERMINAR NIVELES DE OVIPOSICION DE *Anastrepha fraterculus* (Wied.) SOBRE FRUTA Y DISPOSITIVOS ARTIFICIALES

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Abstract–Resúmen

TRIALS TO DETERMINE LEVELS OF OVIPOSITION OF *Anastrepha fraterculus* (Wied.) ON FRUIT AND ARTIFICIAL OVIPOSITION DEVICES.

The current work was conducted in order to determine the level of oviposition of *Anastrepha fraterculus* on fresh mangos and artificial oviposition devices.

Four trials were carried out during which freshly harvested fruit was exposed to wild South American adult fruit flies. The following viable ovipositions/day/kg of fruit (viable ovipositions = emerged adults) were established: 79.16, 71.25, 83.6 and 60.6. The trials were carried out at 25°C-28°C and 60%-80% relative humidity.

Beginning with the first generation one trial was carried out which confirmed the acceptance of sexually mature females to oviposit on red oviposition devices consisting of wax-covered cloth. However, red plastic containers with holes of 0.5 mm did not produce good results.

ENSAYOS PARA DETERMINAR NIVELES DE OVIPOSICION DE *Anastrepha fraterculus* (Wied.) SOBRE FRUTA Y DISPOSITIVOS ARTIFICIALES.

El presente trabajo fue conducido para determinar el grado de oviposición de *Anastrepha fraterculus* sobre mangos frescos y dispositivos de oviposición.

Se realizan 4 ensayos en los cuales se expone fruta fresca recièn cosechada a adultos silvestres de la mosca sudamericana de la fruta determinàndose 79.16, 71.25, 83.6 y 60.6 posturas viables/dìa/kg de fruta (posturas viables = adultos emergidos). Los ensayos se realizan a 25°C-28°C and 60%-80% de humedad relativa.

A partir de la primera generación se realiza 1 ensayo, determinàndose la adaptabilidad de las hembras sexualmente maduras para ovipositar sobre mallas parafinadas de color rojo. Sin embargo los vasos plàsticos de color rojo con agujeros de 0.5 mm no mostraron buenos resultados.

1. INTRODUCCION

En nuestro pais *Anastrepha fraterculus* Wiedemann esta considerada como plaga limitante de nuestras exportaciones debido a las restricciones cuarentenarias impuestas por mercados mundiales importantes. Su distribuciòn en nuestro pais alcanza zonas exportables de frutas y/o con potencial para ello.

Estudios en el Perù sobre la aplicaciòn de la Tècnica del Insecto Estèril en el control de la mosca sudamericana de la fruta *Anastrepha fraterculus* fueron conducidos por J. Gonzales et al. en 1971 [1]. Los trabajos de laboratorio fueron conducidos entre otros objetivos para determinar que la mortandad al estado de huevo fue de 43.5 %. Se demostrò que la tela de nylon de color rojo fue la màs adecuada para utilizarse como superficie de oviposicion, en comparaciòn con las de otros colores tales como verde, amarillo o blanco.

2. OBJETIVO

Se busca determinar el nivel de infestaciòn a partir de hembras de *Anastrepha fraterculus* sobre frutos de mango de la variedad Haden recièn cosechados, en condiciones de laboratorio. Tambièn se estudia la adaptabilidad de èstas para ovipositar sobre mallas parafinadas de color rojo o sobre vasos de plàstico rojos con agujeros de 0.5 mm de diàmetro.

3. MATERIALES Y METODOS

Todos los ensayos fueron conducidos en condiciones de laboratorio a 25° - 28°C y 60% - 80% de humedad relativa.

Los adultos de *Anastrepha fraterculus* progenitores fueron obtenidos de muestreos dirigidos de guayabas en huertos de La Molina, Lima. Las muestras fueron pesadas y colocadas en cajas de recuperaciòn. Las pupas obtenidas fueron pesadas y cuantificadas. El nivel de infestaciòn es expresado como cantidad de pupas por kilogramo de fruta muestreada. Los resultados se muestran en la tabla N° 01.

TABLA N° 1:   RESULTADO DEL MUESTREO DE GUYABAS

PESO DE MUESTRA (Kg)	N° DE PUPAS RECUPE-RADAS	PUPAS/Kg DE MUESTRA	PESO DE PUPAS (mg)	EMERGEN-CIA DE ADULTOS (%)	N° DE A. fraterculus
0.5	200	400	11.00	98.00	194
4.0	1119	280	12.22	89.90	1006
11.3	1832	162	14.70	94.97	1735
9.7	4415	455	13.13	96.08	3958
3.5	3278	937	11.50	94.20	3069
TOTAL: 29	TOTAL: 10844	PROMEDIO : 446.8	PROMEDIO : 12.51	PROMEDIO: 94.63	TOTAL: 9962

Los porcentajes de emergencia se refieren a adultos de *A. fraterculus* y una mìnima cantidad de otras especies de moscas

3.1. Ensayos de oviposiciòn sobre fruta, en laboratorio

Se realizaron 04 ensayos para determinar el grado de infestaciòn por parte de hembras silvestres obtenidas del muestreo de guayabas.

En jaulas de 0.67 x 0.36 x 0.60 m. se tratò de mantener una densidad menor a 0.035 adultos/cm^3 Las hembras sexualmente maduras tuvieron entre 1 y 2 meses de edad. Los mangos expuestos fueron de la variedad Haden y habìan sido cosechados recièntemente. Previamente a la exposiciòn se les aplicò 1.0 KGy de radiaciòn gamma en un Gammacell 220

del Instituto Peruano de Energìa Nuclear con el fin de eliminar posibles inmaduros. El tiempo de exposiciòn fluctuò entre 6 y 8 dìas y se le dio un fotoperìodo de 09 horas luz mediante 02 tubos fluorescentes de 40 watts cada uno. Los resultados de los cuatro ensayos muestran que a partir de 2,946 moscas silvestres se alcanzaron 79.16 posturas que completaron el ciclo/dìa/Kg de mango; de 3,574 se obtuvieron 71.25; de 2,720 se consiguieron 83.6 y de 3,196 adultos 60.6 posturas que completaron el ciclo/dìa/Kg de mango expuesto.

Mediante muestreos de los adultos se determinò que la relaciòn de sexos fue aproximadamente 1:1, motivo por el cual podrìamos considerar que la cantidad de hembras existentes podrìa ser la mitad del total de adultos utilizados en los ensayos.

Los resultados se muestran en la tabla N° 2.

TABLA N° 2 :   RESULTADO DE LAS INFESTACIONES SOBRE MANGO

N° DE ADULTOS	EDAD DE ADULTOS (DIAS)	TIEMPO DE EXPOSIC. (DIAS)	N° PUPAS RECUP.	PESO DE PUPAS (mg)	PUPAS/ Kg DE MANGO	HUEVOS VIABLES/ DIA/Kg DE MANGO	EMERG. ADULTOS (%)
2946	34	6	950	10.39	475.0	79.16	78.84
3574	42	6	855	7.43	427.5	71.25	67.25
2720	55	8	1338	15.37	669	83.6	63.00
3196	63	8	971	11.41	485.5	60.6	60.04
TOTAL 12436	PROM. 48.5	PROM. 7.0	TOTAL 4114	PROM. 11.15	PROM. 514.25	PROM. 73.65	PROM. 67.28

3.2. Ensayo de oviposiciòn sobre dispositivos artificiales

Se realizò un ensayo con el objeto de demostrar la disponibilidad de oviposiciòn de 1500 hembras de *Anastrepha fraterculus* de la primera generaciòn obtenidas a partir de infestaciones sobre mango en laboratorio (Aproximadamente 3000 adultos) a partir de los primeros 15 dìas de vida, durante 02 semanas consecutivas sobre malla parafinada color rojo cuya superficie de oviposiciòn de 3,960 cm^3 fue impregnada de jugo de mango e iluminada con 02 tubos fluorescentes de 40 watts cada uno con un fotoperìodo de 08 horas luz. Las dimensiones de la jaula fueron 0.66 m x 0.31 m x 0.60 m. Cada hora con la ayuda de un pincel se limpiaba la malla con el objeto de retirar los huevos que se habìan quedado pegados en ella. Diariamente se tomaron muestras de 100 huevos para colocarlos sobre papel filtro negro humedecido en placas petri con el fin de determinar los porcentajes de eclosiòn.

Los resultados muestran que se lograron colectar 98,032 huevos en total (Para esta determinaciòn se considerò 25,000 huevos por cc.). El promedio de eclosiòn fue bastante bajo, 13.95 %. Esto se explica por el hecho que gran parte de èstos se quedaban pegados en la superficie de oviposiciòn causando mortandad por efectos de deshidrataciòn. Los resultados se muestran en la tabla N° 3.

En la misma jaula se colocaron 5 vasos de plàstico de color rojo con agujeros de aproximadamente 0.5 mm de diàmetro, cuyo interior fue tapizado con papel filtro de color

negro humedecido y todo el dispositivo invertido sobre placas petri con agua. Los vasos fueron revisados diariamente no habièndose observado buena disponibilidad de las hembras para ovipositar sobre este dispositivo artificial.

4. CONCLUSIONES

De los ensayos realizados podemos concluir, se tomaron muestras dirigidas de guayabas en huertos de La Molina, Lima haciendo un total de 29 Kg. Se recuperaron en el laboratorio 10,844 pupas con un peso promedio de 12.51 mg. El nivel de infestaciòn fue del orden de 446.8 pupas/Kg de muestra, habièndose obtenido un promedio de emergencia de adultos de 94.63% del cual se recuperaron 9,962 adultos de *Anastrepha fraterculus*.

TABLA N° 3: RESULTADOS DE OVIPOSICIONES SOBRE MALLA PARAFINADA ROJA

ORDEN DE OVIPOSICION (DIAS)	HUEVOS COLECTADOS	ECLOSION (%)	HUEVOS ECLOSIONADOS
1°	4208	7.6	320
2°	3725	6.4	238
3°	4667	12.7	593
4°	10408	19.6	2040
5°(*)			
6°	10037	15.8	1586
7°	9545	22.7	2167
8°	11521	20.3	2339
9°	12320	9.7	1195
10°	5820	13.5	786
11°	10812	18.6	2011
12°(*)			
13°	8222	8.3	682
14°	6747	12.2	823
	TOTAL: 98032	PROMEDIO: 13.95 %	TOTAL: 14780

(*)Dìas que no se realizaron colectas.

Con los adultos obtenidos del muestreo se realizaron ensayos de infestaciòn sobre mangos recièn cosechados. La cantidad de adultos en los cuatro ensayos fue 12,436 con un promedio de edad de 48.5 dìas. El tiempo de exposiciòn de la fruta a las hembras de *A. fraterculus* fue en promedio 7 dìas habièndose recuperado en total 4,114 pupas con un peso promedio de 11.15 mg. El nivel de infestaciòn en laboratorio nos indica en promedio 514.25

pupas/Kg de mango expuesto lo cual significa 73.65 posturas viables /dìa/Kg de mango. La emergencia de adultos fue 67.28 %.

El ensayo para determinar la disponibilidad de las hembras sexualmente maduras para ovipositar sobre dispositivos artificiales nos indica que èstas prefirieron a la malla parafinada de color rojo en lugar de vasos plàsticos con agujeros de 0.5 mm de diàmetro. Sobre la malla se colectaron 98,032 huevos en un perìodo de 2 semanas con un porcentaje de eclosiòn muy bajo, 13.95 %.

REFERENCIA

[1] GONZALES, J. et al. Estudios sobre la aplicaciòn de la tècnica de machos estèriles en el control de la mosca sudamericana de la fruta, *Anastrepha fraterculus* Wied. Revista Peruana de Entomologìa volumen 14, N° 1, 1971. 66-86

AVANCES SOBRE LA CRIA ARTIFICIAL DE
Anastrepha fraterculus (Wied.) (DIPTERA: TEPHRITIDAE)
EN COLOMBIA

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Abstract–Resúmen

PROGRESS ON THE ARTIFICIAL REARING OF *Anastrepha fraterculus* (Wied.) (DIPTERA: TEPHRITIDAE) EN COLOMBIA.

With the purpose to evaluate post-harvest quarantine treatments for fruits in Colombia, we have been stablished experimental colonies of fruit flies (DIPTERA: TEPHRITIDAE) at the Colombian Agricultural Institute (ICA), plant quarantine Laboratory Ibagué (Tol.) at 24°C, 70-80% RH, and 10 hr light the procedures and results refers only to *A. fraterculus* from september 1994, to september 1996. The first adults, obtained from *Coffea arabica* L. cherries, were initially multiplied in fruits and later on artificial diet. The handling procedures, diets, and data colection are adaptation from those established by USDA-ARS 1981, Celedonio et al. 1989, Gonzalez et al. 1971, Martínez et al. 1987, and others, used for *Anastrepha* spp.

The average percentages of recuperation between stages have been hattching 66.0 ± 1.0; first to third instar larvae 28.12 ± 14.4; third instar larvae to pupae 81.80 ± 3.0; pupae - to adult 75.82 ± 3.4. Adicional data related to partial mortality of the stages are also discussed. The average recuperation from eggs to third instar larvae of 17,57%, and from eggs to adults emerged of 9.5 ± 4.9 are low and indicate the necesity of doing basic research to improve the procedures.

AVANCES SOBRE LA CRIA ARTIFICIAL DE *Anastrepha fraterculus* (Wied.) (DIPTERA: TEPHRITIDAE) EN COLOMBIA.

Para evaluar tratamientos cuarentenarios de postcosecha, se han establecido colonias experimentales de moscas de las frutas en el Laboratorio de Cuarentena Vegetal del Instituto Colombiano Agropecuario ICA en Ibagué a condiciones ambientales de 24°C, 70-80% HR, y 10 HR luz. Los resultados se refieren a *A. fraterculus* y corresponden al período septiembre de 1994 a septiembre de 1996. Los adultos iniciales obtenidos de *Coffea arabica* L. se multiplicaron en frutas hasta tener una dieta adecuada para el desarrollo de larvas. Los procedimientos para el manejo, preparación de dietas y toma de datos se adaptaron de los establecidos para otras especies del mismo género.

Los promedios del porcentaje de recuperación entre los estados de desarrollo observados durante el período fueron: eclosión 60,0 ± 11.0; primero a tercer instar laval 28,12 ± 14,4; tercer instar a pupa 81.80 ± 3.0; pupa a adulto 75,82 ± 3.4; se presentan adicionalmente porcentajes parciales de pérdidas en los estados de larva, pupa y adultos y otros datos. La recuperación de huevo de larvas de tercer instar de 17.57 ± 8.8 y de huevo a adultos que alcanzan madurez sexual de 9.54 ± 4.9 son indicativos de la necesidad de investigar para mejorar la eficiencia del procedimiento.

1. INTRODUCCION

Con el propósito de cumplir con los requisitos cuarentenarios exigidos por las entidades sanitarias para exportar frutas y hortalizas se inició en Colombia desde 1992, un proyecto nacional financiado con presupuesto oficial, bajo la responsabilidad de la División de Sanidad Vegetal del Instituto Colombiano Agropecuario ICA. Para el funcionamiento de este proyecto se construyeron tres (3) laboratorios de seguridad cuarentenaria, para cría de moscas de las frutas y con los equipos necesarios para evaluar tratamientos cuarentenarios de postcosecha,

uno de estos está localizado en Ibagué, departamento del Tolima, en donde se ha avanzado en el desarrollo de métodos de cría de *Anastrepha obliqua* (Macquart) *A. fraterculus* (Wied) y proximamente de *Ceratitis capitata* (Wied). A pesar que la cría de moscas de las frutas con fines experimentales y para la aplicación de la técnica del insecto esteril (TIE) ha avanzado notablemente en varios países, esta es la primera oportunidad que se adapta la técnica en Colombia. En esta publicación se incluyen solamente los procedimientos y resultados de la cría de *Anastrepha fraterculus*. Los procedimientos empleados son una adopción de los utilizados para cría de *A. obliqua* y *A. ludens* en Weslaco Tx [1, 3], Moreno D.S. 1992, comunicación personal, *A. suspensa* en Miami Fl. [2], *A. fraterculus* en Perú [4] y en Brasil [5], entre otros.

2. MATERIALES Y METODOS

2.1. Localización

Los trabajos se han desarrollado en el Laboratorio de Sanidad Vegetal del Centro de Diagnóstico del ICA en Ibagué, departamento del Tolima (latitud 4° 28'N, longitud 75°17'W), los cuartos de cría se mantienen a condiciones ambientales con temperatura promedio de 24°C (_min 23°, y x max 26°), HR 70-80% y 10 hr luz.

2.2. Origen de la cepa y cría en frutas

Los primeros adultos se obtuvieron de cerezas de café arábigo (*Coffea arabica* L) colectados a 1300 msnm, que se mantuvieron en bandejas de poca profundidad, sobre una capa de vermiculita húmeda durante una semana, cuando se revisaron para separar las larvas de tercer instar y las pupas. Estas se dejaron en frascos de 500 cc hasta el inicio de la emergencia de adultos. Estos se pasaron a jaulas de aluminio angular de 30 x 30 x 30 cm con caras laterales recubiertas de malla y se alimentaron con levadura hidrolizada y azúcar (1:3) y agua.

Como substrato inicial para el desarrollo de las larvas se expusieron frutas dentro de las jaulas a partir de los 14 días de edad de los adultos. Las frutas infestadas se colocaron en cajas aireadas a temperatura ambiente durante 8 a 10 días, cuando se separaron por lavado las larvas de 3 instar (L3). Estas se pasaron a cajas plásticas de 10x10x5,5 cm con vermiculita húmeda (300 ml de vermiculita 60 ml de agua) como medio para pupación. Las cajas con las pupas (P) de 12-13 días de edad se colocaron dentro de las jaulas para emergencia. Durante 1993-95, se utilizaron tomate de árbol tamarillo (*Cyphomondra betaceae* Send) papaya (*Carica papaya* L.) y curuba (*Passiflora mollisima* H.B.K.). En cada exposición se utilizaron muestras en promedio de 0.7 kg y se tomaron los siguientes datos: número y peso de frutas, L3 obtenidas, pupas totales (PT) no emergidas (P no emerg) y pupas emergidas parcialmente (P emerg Pr), total de machos y hembras obtenidas (Ad.t) y muertos en la primera semana para apreciar el número de adultos que están próximos a alcanzar la madurez sexual (Ad. Rep).

En las últimas etapas de este proceso y mientras se dispuso de una dieta adecuada para larvas, se usaron sólo papaya y curuba por estar disponibles en el mercado durante casi todo el año a un precio razonable.

2.3. Cria en dieta

Una vez que se dispuso de materiales y procedimientos adecuados se ha continuado con el proceso por métodos artificiales, que se explican a continuación:

2.3.1. Manejo de adultos

Los adultos se mantienen en jaulas de aluminio angular de diversos tamaños con base en lámina de plexiglas transparente y caras restantes con tela malla de nylon de 8 hilos por cm.; una de las caras laterales dispone de una manga de 15-17 cm de diámetro por 25 cm de largo para la manipulación de los insectos y materiales. El número de adulto por jaula varía de acuerdo con las dimensiones, así en jaulas de 20x20x20 cm hasta 300, cen las de 30x30x30 cm hasta 1.700 y en las de 70x50x50 hasta 6.700. Dentro de cada jaula se coloca el alimento para adultos constituido por 40% de levadura hidrolizada (autolyzed brewers yeast, Bioserve Inc); 56% de azúcar; 2% de mezcla de vitaminas (USB. Vanderzant Vitamins fortilication for insects) y 2% de sales Wesson (Bioserve Inc), adicionalmente se coloca azúcar en recipientes separados. El agua se suple en frascos con tapa provista de un hueco circular para el paso de un algodón dental, el agua y el alimento se cambian una vez por semana o cuando es necesario por crecimiento de hongos o hidratación; con la misma frecuencia se retiran los adultos muertos. Las condiciones del cuarto de cría son las ya señaladas los adultos se mantienen con un fotoperíodo de 10 horas luz y 14 de oscuridad, la luminosidad se origina en lámparas fluorescentes luz día de 20W colocadas a 10 ó 15 con sobre las jaulas.

2.3.2. Recolección e incubación de huevos

Los huevos (H) se recolectan en medios hemisfericos hechos con tela de algodón de 16 hilos/cm. Impregnada en una mezcla de parafina y vaselina (1:1) y colores vegetal amarillo y rojo soluble en grasa que se agregan cuando la mezcla está caliente hasta lograr el tono deseado. Para dar la forma a la tela parafinada se usa un molde de madera. El medio parafinado se coloca sobre un anillo de PVC del mismo diámetro del molde y se asegura con una banda elástica de 3 mm de ancho. Los medios se asperjan con solución de benzoato de sodio (BeNa) al 0.02%, se coloca por dentro una esponja celulosa de 1.0 cm de grosor humedecida, así preparados se colocan dentro de las jaulas por 8 horas. La recolección se hace dos veces por semana desde los 13 hasta los 40 días de edad de los adultos. Para la separación de los huevos, se utiliza un aspersor manual con solución de BeNa, se lavan dos veces y se dejan decantar en una probeta, para obtener la cantidad por volumetría (1 ml - 20.000 hy). Luego se colocan sobre papel de filtro y agua destilada y se mantienen en cajas petri de vidrio que se guardan dentro de una caja plástica cerrada y provista de una esponja húmeda en la base, en donde se incuban por 3 ó 4 días, se pasan a dieta cuando se observa, 50 a 60% de eclosión en una submuestra mantenida en iguales condiciones.

2.3.3. Desarrollo de larvas

Las larvas se alimentan de dieta artificial que por la naturaleza de los componentes corresponde al tipo merídica. Desde el inicio de la producción en dieta en 1994, hasta la fecha se han evaluado tres modalidades que han variado esencialmente en la calidad del material portador.

La usada en el último año tiene la siguiente composición por ciento: Torula tipo B. (bioserve Inc): 6.0; azúcar = 6.0; germen de trigo = 6.0; colesterol 0.05; agar - agar 3.0; metil paraben 0.1; BeNa 0.1; agua 70 a 80 y HCL = 0.5 ml para mantener el pH entre 3.7 y 4.0. La dieta se coloca en cajas plásticas de dimensiones variables con tapas provistas de un espacio cubierto con malla de fibra de vidrio para aireación, la capa de dieta usualmente es de 1.5 cm de espesor y se calculan cinco larvas por mg de dieta. Las larvas se retiran de la dieta por lavado a los 8 ó 10 días, una vez que se han formado las primeras pupas. En este momento se separan y cuentan las L3 desarrolladas y se desechan las de menor tamaño. Las primeras se pasan a vermiculita húmeda para preparación.

2.3.4. Mantenimiento de pupas

Las pupas se mantienen en cajas de 10x10x5.5 cm en las cuales se coloca vermiculita húmeda, a razón de 300 ml más 60 ml de solución de BeNa al 0.2%; para incrementar la humedad ambiental se mantienen en una cámara cubierta con tela plastificada y se dejan durante 13 días cuando se separan para hacer una evaluación del número, calidad y peso, y solo las aparentemente viables se pasan a jaulas de emergencia en vermiculita húmeda. Ocho o diez días después se evalúan las pupas emergidas, no emergidas, emergidas parcialmente, adultos totales y muertos durante este período.

2.3.5. Datos

Con los datos tomados durante los procesos anteriores se calculan los porcentajes de sobrevivencia y motalidad de cada uno de los estados y los de recuperación o conversión entre los distintos estados, causas de pérdidas parciales en los estados de larva (L3 no formadas o muy pequeñas) de pupa (P no emergidas o emergidas parcialmente) y adultos muertos en la primera semana. Los promedios que se presentan corresponden al período Septiembre de 1994 a Septiembre de 1996 o sea a 25 meses.

3. RESULTADOS Y DISCUSION

3.1. Cría en frutas

La multiplicación de los adultos recuperados de café en fruta no es un método ventajoso, pero fue útil como primer recurso, por no disponer de todos los elementos para iniciar la cría artificial. De las frutas utilizadas no es hospedante natural la papaya; la mora es severamente atacada cuando se cultiva en la zona cafetera marginal alta (1.700 msnm), la curuba y tomate de árbol, se han identificado como hospedantes pero el daño directo es esporádico cuando se cultivan en la zona marginal cafetera alta. No se ha de terminado infestación en ninguna de las tres especies cuando crecen por encima de 2.200 msnm en donde *A. fraterculus* no se ha establecido. La atracción de los insectos hacia las frutas fue similar en todos los casos, pero hubo diferencias en cuanto a la aceptación como sitio para oviposición. En tomáte de árbol, se encontraron numersos huevos fuera de la fruta, posiblemente por la resistencia del epicarpio. La mora, fue muy aceptada, y se encontraron de uno a tres huevos por fruta, que pueden desarrollarse exitosamente, pero la recuperación real de la L3 fue baja por efecto de la descomposición rápida de la fruta en postinfestación. La papaya y la curuba presentaron resultados similares, pero la última, se descompone menos rápidamente y por esta razón fue más utilizada. Los porcentajes de sobrevivencia entre los estados fueron similares en mora, papaya y curuba, pero hubo gran dfierencia en el indice de infestación (L3 kg), el

TABLA 1. PORCENTAJE DE SOBREVIVENCIA, MORTALIDAD PARCIAL DE PUPAS, INFESTACION Y RECUPERACION DE A. *FRATERCULUS* EN FRUTAS INFECTADAS EN LABORATORIO (24°C, 70-80% H.R.). IBAGUÉ (COL.) 1993-1995.

Fruta		Sobrevivencia %			Mortalidad pupas		Infestación	Recuperación
		3-P	-Ad	d-Rep	No.emerg.	Emg parc.	L3/kg	L3-AdRep.
S. betaceae	31	29.05	28.7	47.4	4.6	0.0	17.2	24.5
R. glaucus	80	79.1	67 8	76.3	24.9	4.0	58.8	38.7
C. papaya	249	79.1	57.6	80.4	36.6	4.7	218.8	38 5
P. mollisima	208	81.2	67.9	79.1	23 5	7.4	413.8	47.9

L3=Larvas de tercer instar; P= pupas; Ad= Adulto emergido; AdRep= Adultos reproductivos
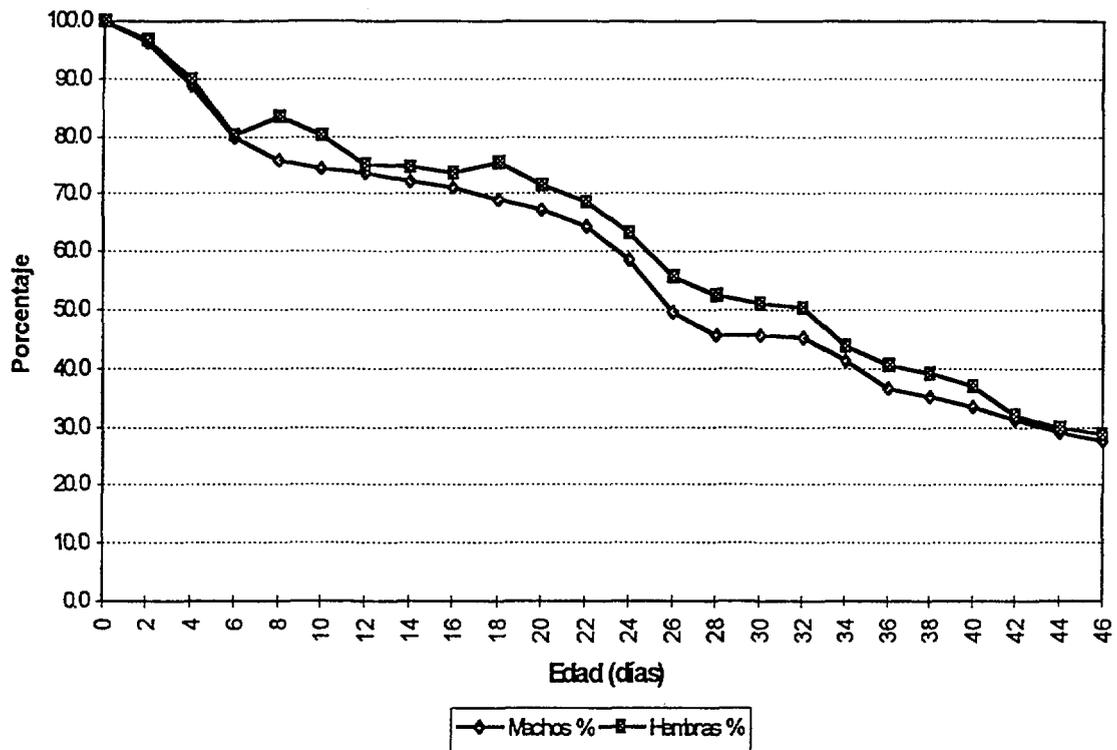
FIGURA 1. SOBREVIVENCIA DE ADULTOS DE *A. FRATERCULUS* OBTENIDOS DE CURUBA (*P. MOLLISIMA*) EN CONDICIONES DE LABORATORIO (24°C, 70-80% HR). IBAGUÉ (COLOMBIA) 1994.
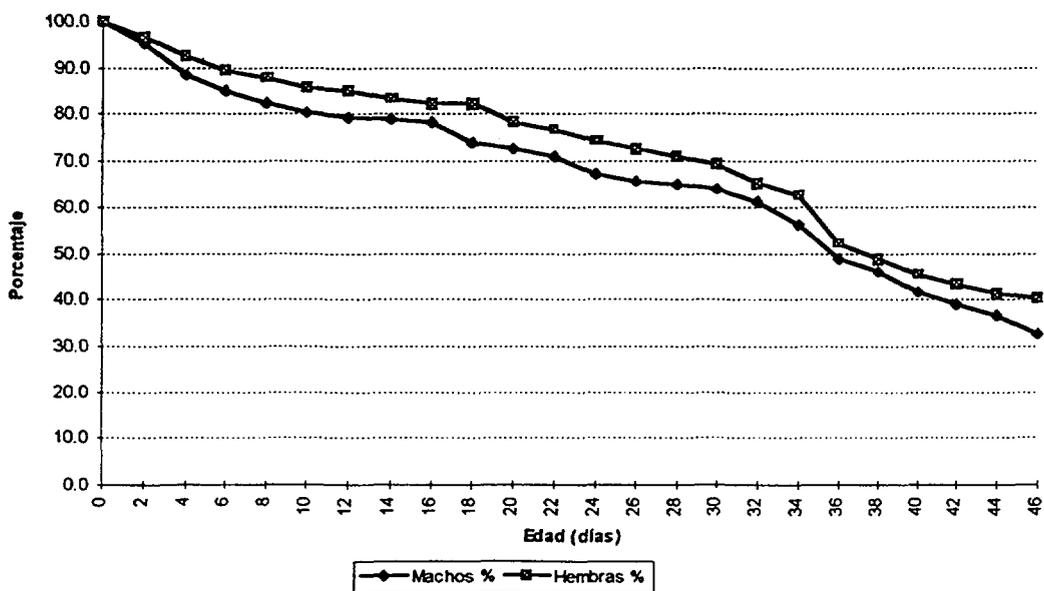

FIGURA 2. SOBREVIVENCIA DE ADULTOS DE *A. FRATERCULUS* OBTENIDOS DE PAPAYA (*Carica papaya*) EN CONDICIONES DE LABORATORIO (24°C, 70-80% HR) IBAGUÉ (COLOMBIA), 1994.

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cual fue muy alto cuando se uso curuba. En todos los casos se observó alta mortalidad de adultos durante los primeros 80 días y solo un porcentaje muy bajo alcanzó la madurez sexual. Los resultados globales de algunos de los índices de producción se presentan en la Tabla 1. Las curvas de sobrevivencia de adultos obtenidas de papaya y curuba, promedio de cuatro repeticiones en cada caso se presentan en las figuras 1 y 2.

La tendencia indica una mejor adaptación y longevidad de los adultos obtenidos de papaya, y que puede estar relacionada con una nutrición más completa de las larvas. La relación de sexos fue de 1:1 en todas las frutas y la duración del ciclo de oviposición a emergencia de adultos fue en promedio de 27 a 30 días. El proceso es muy costoso, por el valor de las frutas y mano de obra y difícil de manejar por la proliferación de hongos de almacenamiento y drosofilas especialmente. A pesar de las desventajas puede ser útil en circunstancias similares a cuando se necesita producir pocas cantidades de insectos para fines de investigación.

4. CRIA EN DIETA

4.1. Oviposición

Las cantidades de huevos recolectados han sido proporcionales en las cantidades de adultos mantenidos en jaulas, pero el incremento es indicativo de la adaptabilidad de la especie a las condiciones de laboratorio. Las condiciones reales (1 ml: 20.000) se presentan en la Figura 3 y son indicativas de la adaptabilidad de la especie a las condiciones de cria artificial, las cantidades durante el período septiembre de 1994 a mayo de 1995 fueron muy pequeñas y por esto son indetectables en la gráfica.

4.2. Fertilidad y recuperación de larvas de tercer instar (L3)

Los porcentajes de eclosión o fertilidad y de conversión de L1 a L3 se presentan en la Figura 4. Durante el proceso, estas son las dos etapas en las cuales se observan las mayores pérdidas. El porcentaje promedio de eclosión durante todo el período considerado fue de 66.0 + 11.0 (min 48.0 y max 85.0). La recuperación de L3 a partir de L1 fue en promedio de 28.12%+14.4 (Min 7.0, Max 50).
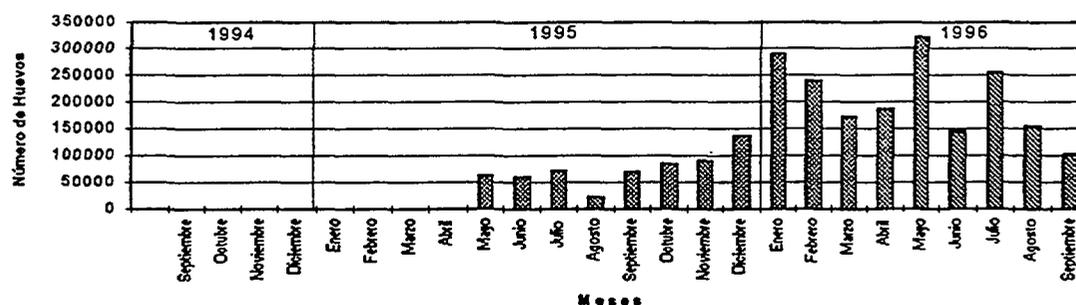

FIGURA 3. PRODUCCIÓN DE HUEVOS (MILES) DE A. FRATERCULUS CONDICIONES DE LABORATORIO 24°C 70-80% HR. IBAGUÉ (COLOMBIA)
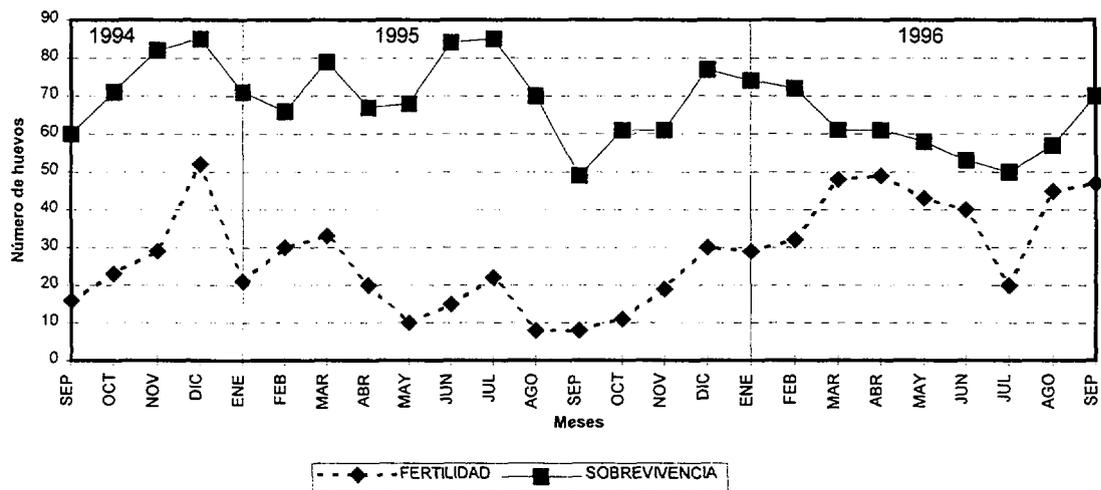
Figura 4. FERTILIDAD Y SOBREVIVENCIA DE LARVAS DE PRIMER INSTAR DE *ANASTREPHA FRATERCULUS* EN DIETA ARTIFICIAL. IBAGUÉ (COL) 24°C, 70-80% HR.

La baja fertilidad, puede ser el resultado de la influencia de las condiciones y manejo de adultos, y/o del manejo de los huevos, pues a pesar de que se tienen muchos cuidados, el proceso implica daños directos. La gran mortalidad de larvas de primer y segundo instar, está determinada casi esencialmente por la naturaleza de la dieta y menor grado por las condiciones ambientales pues las del laboratorio en donde se hizo el trabajo corresponden a las de la zona de mayor adaptación en condiciones de campo. No hubo influencia detectable. de las tres dietas utilizadas, sobre la sobrevivencia de larvas.

4.3. Recuperación de pupas

La sobrevivencia de L3 durante los tres años de observación se presentan en la Figura 5A. En el paso de L3 a pupa se ha observado una recuperación promediode 81.80 + 3.0% (mínimo 72.0% y máximo 93.0%). Esto indica que aproximadamente el 18% de las L3 bien formadas que pasan a vermiculita, se pierden durante el proceso de pupación. Se agrega a ésta, una pérdida del 7.42% ± 8.9 de larvas de tercer instar que se desarrollan anormalmente y que por ser muy pequeñas se desechan en el proceso, de solución de L3. El peso promedio de las pupas es de 13.19 + 0.92 mg.

4.4. Emergencia de adultos

La emergencia de adultos se inicia entre los 15 y 17 días después de formada la pupa; puede observarse durante 3 ó 4 días, aún cuando el 80% de los adultos emergen en los dos primeros días, especialmente en las horas de la mañana. Teniendo en cuenta que antes de la emergencia se separan, las pupas con apariencia viable, se ha observado un promedio total de emergencia del 75.82%± 3.4 (Mínimo 39% y Máximo de 97%). El porcentaje restante de pupas se distribuye en pupas no emergidas y emergidas parcialmente que representan en promedio el 21.36 y 2.89% respectivamente. Los promedios mensuales de porcentaje pupas se desarrollan normalmente y que originan adultos se presentan en la Figura 5B.

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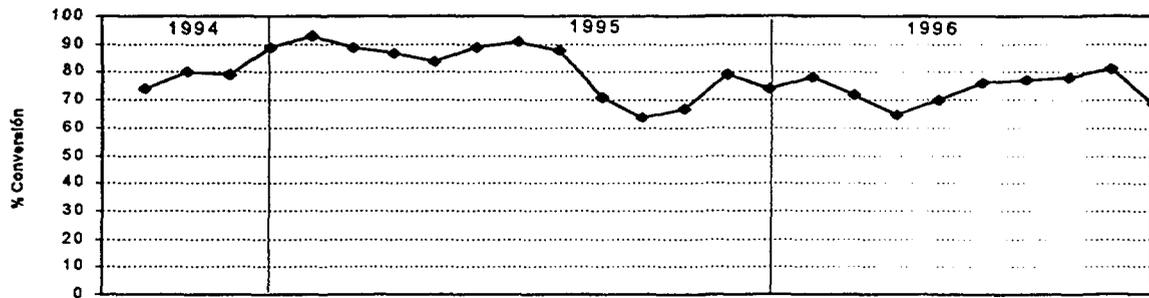
FIGURA 5A. CONVERSIÓN (%) DE LARVAR DE TERCER INSTAR DE *A.FRATERCULUS* EN PUPAS. CONDICIONES LABORATORIO (24°C, 70-80% HR)IBAGUÉ, COLOMBIA

Meses
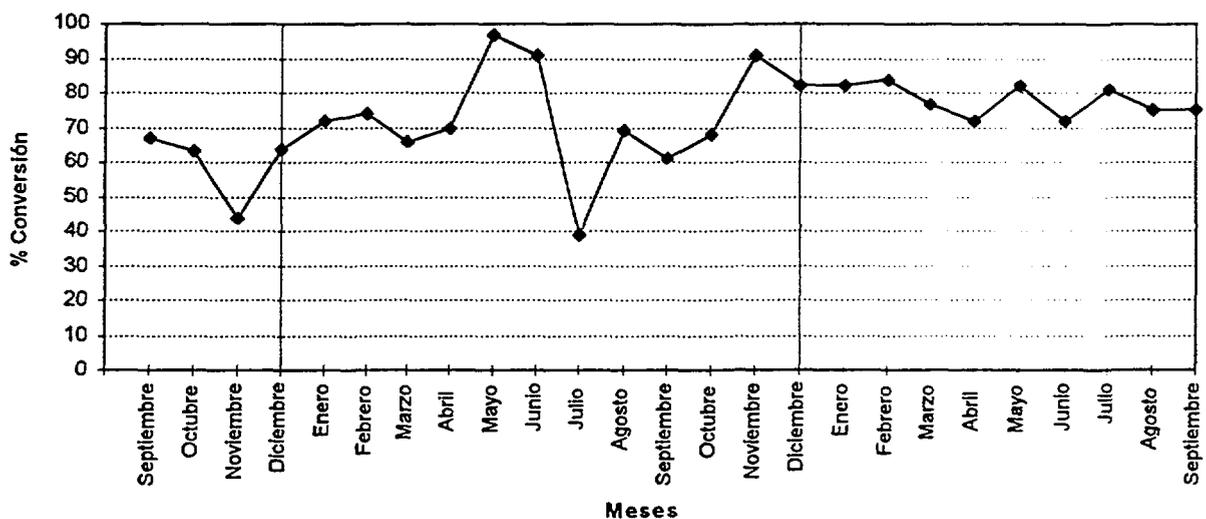
FIGURA 5B. CONVERSIÓN (%) DE PUPAS DE A. FRATERCULUS EN ADULTOS. CONDICIONES DE LABORATORIO (24°C, 70-80% HR) IBAGUÉ, COLOMBIA.

4.5. Mortalidad de adultos

La mortalidad promedio de adultos durante la primera semana es de 35% ±2.9 y es mayor para machos que para hembras. A partir de 8 a 10 días de edad la mortalidad es gradual y se alcanza el 50% entre 40 y 45 días, cuando las jaulas se desechan.

4.6. Ciclo biológico

Bajo las condiciones y manejo utilizados la duración promedio en días para los estados de desarrollo observados fueron: huevos 3.8 ± 0.9; larva (L1-L3) 11.98 ± 3.0; pupa 17.2 ± 2.1, para un total de 31.8 ± 2.3. El período de preoviposición ha sido de 12 a 13 días y se mantiene la relación sexual 1:1. Los datos anteriores son similares a los obtenidos por González, [4], y por Machado, [6] , aún cuando estos corresponden a estudios realizados a 25°C.

4.7. Recuperación Porcentual de los estados de desarrollo

Estos se calcularon con base en los individuos obtenidos de cada estado (L1, L2 P, Ad) respecto al número de huevos recolectados por semana y se obtuvieron los siguientes: huevo a larva de primer instar (fertilidad) 67.49 ± 10.57; huevo a larva de tercer instar 17,57 ± 8.8; huevo a pupa 13.98 ± 7.40; huevo a adulto emergidos 9.54 ± 4.92; huevo a adulto reproductivo 9.544± 4.9. Estos son indicativos del rendimiento del proceso y señalan que las mayores pérdidas se presentan durante el período de incubación y en durante el proceso de desarrollo de los instares larvales. En la literatura disponible no hay muchos datos que pueden servir de comparación de estos resultados, pero son indicativos de la necesidad de investigar especialmente en superficies para oviposición, manipulación de huevos y mejoramiento de dietas pra larvas.

Teniendo en cuenta la importancia económica y filogenética de *A. fraterculus* para Colombia y para todo el Neotrópico es importante el continuo desarrollo del proceso hasta alcanzar mejores resultados a menor costo.

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REARING OF *Anastrepha fraterculus* (Wiedemann)

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Abstract

REARING OF *Anastrepha fraterculus* (Wiedemann).

A few attempts were conducted to establish basic needs, materials, conditions and procedures for artificial rearing of *Anastrepha fraterculus*, henceforth AF. A brief summary will be presented based on published and personal information.

1. REARING OF *Anastrepha fraterculus*

1.1. Egg (oviposition)

This phase is currently believed to be the most critical in the rearing of AF, either to obtain a viable egg (fertilized) or a sufficient number of eggs, due to cost and lack of simple methods. To obtain the egg itself is not a problem, but how to use it to generate larva has, so far, been very complex and difficult.

Rearing procedures used in Pelotas, Brasil, are summarized as follows:

"Artificial fruits" were used as oviposition substrates. These substrates were made with: agar (8,5g), water (350ml), blueberry juice (75ml) and nipagin (4ml). Agar is diluted with cold water, then boiled with constant stirring, then blueberry juice and nipagin are added while the medium is still warm. This liquid is poured out in a half ice tray (ball/round format). In a few minutes the liquid is solidified and can be removed from the tray. A piece of Parafilm (3 x 4 cm) is cut, and the half ball is wrapped, with a maximum expansion of the film. This wrapped substratum is laid on the floor of adult fly cage, with the convex side facing up.

The time that substrate is exposed to flies will depend on the number of eggs desired, egg age, etc. For colony rearing/ maintenance, we expose these substrates for 24 hours and each substrate contains more than 1000 eggs.

1.2. Larva (rearing room at 25°C, 60-70%RH, 16h light)

Egg substrates are removed from the oviposition cage and the remaining parafilm is removed by hand. Two methods are followed: put the substrate immediately on the larval diet, or put the substrate in an incubator for two days (± 26°C) and then put on diet medium. There is no big difference in cost, work or disease incidence.

Components used for one liter of larval diet are: wheat germ, 60g; brewers yeast, 60g; white sugar, 60g; sodium benzoate, 1g. These components are mixed in a 2 liter container. Add 400ml of cold water to this container and mix. Adding 6ml of concentrated (37%) hydrochloric acid and 8ml of nipagin (10%, diluted in alcohol). In another vessel, put 400ml of water and 10g of agar. Heat this mixture up to boiling point with constant stirring. This hot medium is added to the previous mixed ingredients. While still hot, medium is poured out in trays. In a few minutes, this diet mixture is solidified. A layer of diet medium in the trays should be about 2cm thick.

These trays could be used at once or refrigerated (in plastic bags), up to 2-4 weeks. Trays must be sealed with plastic to avoid escape of pre-pupal larvae. Ordinary plastic film and tape or self aderent plastic film could be used for this purpose.

When the first pupae are formed on the diet, the tray should be washed to remove pre-pupal larvae and pupae already formed. The tray contents are poured out into a PVC cylinder with a sieve or into an ordinary large seive (15-20cm diameter) with 1mm mesh and the remaining diet washed with a strong water flow (water temperatures between 10-35°C have no negative effects on larvae or pupae). Pre-pupal larvae of AF seem to be extremely resistant to physical shock and no effect was ever noticed due to handling larvae this way. Larvae and pupae are put in paper towels or on a layer of dry sand or soil in plastic containers. When the first adults emerge, containers are put into adult rearing cages.

An equal number of females and males is desired in the cage (? if necessary). We do not count, but just estimate the proportion. When one sex is superfluous, we add or transfer the excess to another cage.

Adults are fed with a semi-solid diet made with brown sugar and hydrolyzed corn protein, plus honey and water. Cleanness of cages (every two months) is made by washing with water and bleach mixture. Dead adults are removed once a week, at most.

1.3. Other artificial laboratory rearing procedures developed for AF

Mass rearing of AF, in my understanding, has never been conducted in any large project , but in only a few laboratory studies with small research objectives or purposes for rearing, and for only a short period of time.

1.3.1. Adult rearing

Temperatures used were between 25-27°C, relative humidity from 60-70% (Gonzalez 1971). Flies were confined in cages of the Hawaii model, measuring 1,20 x 0,60 x 0,30 m, and later these cages were replaced with another model, Peleg-Simon, with dimensions of 2,0 x 0,60 x 0,35. In the Hawaii cages, three levels of pupae concentration were tested in a cage: 10.000; 12.000 and 15.000 puparia/cage. 12.000 puparia/cage gave the best results, producing nearly 3cc of eggs/day/cage (± 13 eggs/female/day).

Simon et al (1971) used as an oviposition substrate, little balls of parafin (same as are used for *A. ludens* in Mexico) and adults confined in Hawaii cages. Later, they used other methods for oviposition: one wall of the Hawaii cage was coated with parafin and painted red. Similar procedures were done with cylindrical devices hung in the center of cages. The same procedures that had been conducted in Hawaii cages were done with Peleg-Simon cages. In these cages, they tested several colors for the parafin wall. Red was the best.

They found that in both cage models, eggs did not fall down off the parafined screen as easy as in the "moscamed" (medfly) cages. They overcame this problem, shaking the parafined wall three times a day. Eggs were washed in a solution of water with sodium benzoate at 0,5% and incubated for 48 hours at 28°C, before depositing them on the diet.

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They mentioned some data obtained with eggs:

	Original*	Actual
production	1,5	3,5 cc/cage/day
viability	24	45%
egg recuperation/pupae	2,9	5,3%

* is not mentioned this method in the paper.

1.4. Larva

Numerous adaptations of other larval rearing methods were done. They obtained negative results with most of these adaptations. They used the following larval diet: Water 3,0 l; sodium benzoate 7g; chloridric acid 25ml; torula yeast CF-2 300g; carrot power 500g, and pH ajusted to 3.8. No results were mentioned on this larval rearing procedure.

1.5. Pupa

They mentioned that the puparia of these species must be washed to separate them from the diet medium. Puparium are left to dry and deposited on "aserrin" for 10 days.

No results were shown on the emergence, viability, etc. of the pupal stage.

Using the above-mentioned methods, they obtained a set of data on the life cycle of AF, in days: Pre-oviposition 8; incubation 3; larva 9; pupa 12; oviposition 45; adult longevity 85; number of mating for males 12; for females, only one; total number of eggs/fly 416 in 45 days.

1.6. Other artificial oviposition methods/devices used for AF

- half fruits without the pulp (shallowed fruits):   Baker, 1945
- waxed cloth of cylindrical format: Simon, 1968
- half sphere made with agar and red colored: Lopes, 1986
- red sponge coated with parafin: Martins, 1986
- half sphere made with agar, red colored, coated with parafilm: Salles, 1992
- half sphere made with larval diet, coated with parafilm: Salles
- natural fruits (papaya, banana, araca,...):   Malavasi, Salles

Diets used for adults:

- Martins, 1986: Fleischmann yeast 6g; honey 10g; white sugar 35g; "levemil" 1g; hydrolyzed protein 5ml.
- Nascimento, A.S.: brown sugar 150g; white sugar 250g; brew yeast 36g; "sustagen" 3g; "levemil" 3g; honey 60ml; hydrolyzed protein 180g.
- Salles, 1992: brown sugar 100g; hydrolyzed corn protein 50ml; water 300ml; honey 2:1 water in soaked cotton.
- Salles, L.A.B. Brown sugar 100g; hydrolyzed corn protein 50ml..

Diets used for larvae:

- Martins, 1986: Parahydroxybenzoate 2g; sodium benzoate 2g; concentrated hydrochloric acid 8,1ml; formaldehyde 0,6ml; sucrose 45g; yeast 67,2g; crushed sugar cane 135g; water 600ml.
- Gonzales, 1971: water 300ml; sodium benzoate 7g; hydrochloric acid 25ml; torula yeast 300g; carrot power 500g; pH 3,8.
- Nascimento, A.S.: white sugar 300g; brew yeast 300g; wheat germ 300g; nipagin 5g; sodium benzoate 5g; sulfuric acid 21ml; water 1500ml.
- Salles, 1992: wheat germ 60g; brewers yeast 60g; white sugar 60g; sodium benzoate 1g; water 400ml; hydrochloric acid (37%) 6ml; nipagin (10%) 8ml.
- University of Florida/Salles, L.A.B: corn cob grit 220g; brewers yeast 40g; white sugar 60g; wheat germ 30g; sodium benzoate 1,5ml; hydrochloric acid 5ml; pH 3,0.
- Walder, J.M: crushed dried sugar cane 99g; wheat flower 99g; brewers yeast 99g; white sugar 160g; citric or hydrochloric acid 4,6ml; sodium benzoate 2,7g; water 540ml; "terramicina" 250mg.

1.7. General comments

AF adults live 100-150 days in a rearing room/laboratory conditions (25°C, 60-70%RH, 16h photophase). As the adults are so long-lived, it is possible that inbreeding could occur in the population. To avoid possible inbreeding, we exchanged adults among cages and added new wild adults in the cage/population. We do not have any evidence of inbreeding (such as decreasing number of eggs, egg fertility, longevity) up to 19 generations in an isolated population.

Average days of the life cycle of AF in the rearing process in use in Pelotas, Brasil: egg 2 (2-3); larva 15 (11-17); pupa 10 (9-12); life cycle (egg to adult) for female was 30 (26-31) and male 31 (28-35) days.

Comparison of life cycles (days) in rearing conditions between AF (Pelotas) and *A. suspensa* (Florida):

	fraterculus	*A.suspensa*
egg	2	3
larva	15	7-8
pupa	10	11-14
life cycle	30	21-25
adult longevity	100-150	12-16

Egg fertility seems to be the major actual bottleneck. It is highly variable throughout the year. Some time, absolutely no or very few larvae emerged and at other times almost full emergence occurs. Despite numerous tests, observations, and changes of all orders in the process, this situation still happen.

I do not have a clear idea why and how it occurs. There are many possiblities that must be considered and discussed by a group of experts on fruit flies and on insect rearing. It must be resolved before any action toward rearing a large amount of AF can be taken.

A protocol of procedures must be established for rearing AF. All biological phases must have a systematic procedure and their performance investigated. After adjustment of any phase, it should be possible to try rearing AF throughout the entire life cycle, making necessary adjustments to improve efficiency and productivity. I believe that the procedures actually used in Gainesville (Fla. Dep. Agric. Cons. Service) for *A. suspensa* could be an excellent starting point and a guidance for AF. Probably, a combination of procedures (Florida, Mexico, Brasil) could be considered. It seems to me that strict rearing procedure (s) for each phase should be determined. Otherwise, AF could be reared but with poor efficiency with potential problems and a high risk of failures.

Quality control measures must be strictly done and evaluated by a board of professionals in mass rearing of fruit flies.

A key factor is to have and maintain a quality standard of all diet ingredients. For example, due to actual administrative procedures, materials are bought in Pelotas, Brasil by price, not necessarily considering their quality. It is obvious that some ingredients are better or more appropriated than are others. For example, when larval diet ingredients are bad, larvae of many sizes with consequently variable pupal sizes are found in the trays. This must be resolved.

1.8. Basic constraints for colonization and establishment of mass rearing procedures for AF

AF is a fruit fly species restricted to the neotropical region and its is a pest in southern South America. Consequently, there are few entomologists working with AF and "two or three" are dedicated full time to this fly. Obviously, there is a lack of information on its biology, ecology, behavior, population structure, population genetics, etc. Very few and localized surveys of its population structure, behavior, field performace, hosts, pest status, etc. have been done, especially under a systematic and constant protocol.

Is AF the same in different regions, areas, localities throughout South America? Could we use the same rearing methods as a standard procedures for any AF? Based on my personal opinion, it is necessary to work intensively with AF to establish a laboratory adult colony that will produce feasible larvae and adults. To reach this goal, the following problems must be solved, at least.

1.9. Basic problems

- Develop an effective oviposition system.
- Develop an effective egg collection system or device.
- Develop an effective system to obtain high (>70%) egg viability.
- Obtain a nutritive rearing medium that ensures high yields and good quality insects.
- Obtain a nutritive rearing medium for adults and larvae, economically coherent and adapted to developing countries.

1.10. Secondary problems

- Rearing cage for adults. Other cage types used for *Anastrepha* (as in Florida, Mexico) should be evaluated, especially to determine adult survivorship, oviposition, egg viability, ease of collection, etc. Evaluation of cage types and oviposition devices must receive high priority and attention. This is a critical point and phase. In my opinion, it is very dangerous to take short cuts, based on preliminary successes (I did that with AF and I know how hard is to maintain a healthy colony of this species, when you have no material, supplies, quality control, etc. In Pelotas, during three consecutive years, AF was reared with success throughout the year. Suddenly, problems (not yet identified), reduced this rearing to a critical condition with high instability).

- It is fundamental to establish a rearing medium for adults and larvae, based on nutritive values, insect quality, availability of components, and costs. A quality standard and substitute must be defined. A rearing manual with rearing procedures and quality control guidlines must be followed.

- As AF was never mass reared, special quality control procedures for adults must be followed, probably using basic tests already used in Florida and Mexico for other *Anastrepha* species. We don't know if inbreeding, fecundity, viability, etc. does or does not occur in colonized AF.

- Procedures to reproduce AF after we obtain laboratory generation (s) should be followed throught rigorous evaluation of the quality of any phase of entire process.

ECOLOGY, BIOLOGY,
BEHAVIOUR AND CONTROL METHODS

Anastrepha fraterculus (WIEDEMANN)
(DIPTERA: TEPHRITIDAE) IN TUCUMAN, ARGENTINA
(Summary)

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In 1916, Rust recorded *Anastrepha fraterculus*, the "South American fruit fly (SAFF)", in Tucumán [1].

Since 1910, the citrus area in the province has increased rapidly and SAFF has become an important pest. New varieties and management practices were also introduced in the region [2]. Guavas *(Psidium guajava)* and peaches *(Prunus persica)* were the main host fruits of SAFF in Tucumán, but cherimoya *(Anona chirimoya)* and apricots *(Prunus armeniaca)* were also important [3].

After the begining of rains, populations increase in spring and develop mainly on peaches in November and December. Afterwards, SAFF attacks guava, where a peak population is attained in February or March. Guava is the principal wild host of SAFF in Tucumán.

Compared with the forest where guava trees were frequent, citrus orchards covered a small area in the 1920's. Therefore SAFF populations increased in the wild guavas and invaded early oranges and grapefruits afterwards.

Even though high numbers of punctures were observed in citrus skin, few larvae developed. The oils present in the skin kill high number of eggs. Moreover the larvae have to go through the albedo and hardly reach the pulp. Nevertheless the injury produced by the ovipositor allows microorganims to rot the fruit. In 1918, rots produced 50% of damage in fruits which suffered premature ripening and fell.

Only in very thin skinned and overripened fruits, larval development was registered. 3]. Some authors observed larval development in the field but Schult [4], in laboratory tests, found very few eggs and never registered larval development.

Between 1920 and 1945 studies with bait-traps were carried out to establish the seasonal occurrence of the fly and some control measures were tested. Poisoned baits were used against this pest, and biological control by inoculation of parasitoids were also employed. Cages with parasitized pupae were distributed to farmers. The emerged flies were kept within the cage and a sieve allowed the emerged parasites to leave the cage and disperse [4, 5, 6, 7].

In March 1945, *Ceratitis capitata* was captured for the first time in the traps. Since that event, insecticide sprays are increasingly used against this pest mainly in citrus orchards[8].

In the fifties the "citrus tristeza virus"(CTV) wiped out all orange, grapefruit and tangerine orchards which were grafted on sour orange rootstock. They were replaced by lemons, the only tolerant species on sour orange. Some orange and grapefruit varieties were also grown on tolerant rootstocks [9].

Between 1950 and 1975 new studies were carried out in order to establish the importance of *C. capitata* as a new pest.

In 1952-53 trap catches showes SAFF more abundant than medfly [10], when lemons were already the predominant species of citrus.

In 1960-63 the number of grapefruits increased again and medfly became more abundant [9].

Between 1960 and 1980, medflies were predominant in trap catches, probably because of the expansion of citrus orchards [11]. No further studies of this species continued after 1980.

It is not clear if medfly displaced SAFF by competition in citrus orchards, or if the expansion of the citrus area restricted SAFF to the forest where wild guavas and peaches are still present.

The fact is that in my samplings of citrus fruits in Tucumán and other places like La Rioja province, I have never obtained specimens of SAFF.

Several laboratory, semifield and field tests should be done in order to establish the current importance of SAFF in Tucumán.

ACKNOWLEDGEMENTS

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ANTECEDENTES SOBRE LA DETECCIÓN Y ERRADICACIÓN DE LA MOSCA SUDAMERICANA DE LA FRUTA (MSAF), *Anastrepha fraterculus* (WIED.) (DIPTERA: TEPHRITIDAE), DE OASIS DEL EXTREMO NORTE DE CHILE (1929-1964)

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Abstract-Resúmen

BACKGROUND ON THE DETECTION AND ERADICATION OF THE SOUTH AMERICAN FRUIT FLY, *Anastrepha fraterculus* (WIED.) (DIPTERA: TEPHRITIDAE), IN THE EXTREME NORTHERN OASIS OF CHILE (1929-1964).

A history of the detection and eradication of the South American fruit fly (*Anastrepha fraterculus* (Wied.)), in Chile, from 1929 to 1964, is presented.

ANTECEDENTES SOBRE LA DETECCIÓN Y ERRADICACIÓN DE LA MOSCA SUDAMERICANA DE LA FRUTA (MSAF), *Anastrepha fraterculus* (WIED.) (DIPTERA: TEPHRITIDAE), DE OASIS DEL EXTREMO NORTE DE CHILE (1929-1964).

Se entregan algunos antecedentes sobre la presencia y erradicación de la Mosca Sudamericana de la Fruta, *Anastrepha fraterculus* (Wied.) en Chile, entre 1929 y 1964.

1. INTRODUCCIÓN

El género *Anastrepha* Schiner, incluye unas 180/190 especies que se distribuyen en las áreas tropicales y subtropicales del continente Americano, desde el sur de Estados Unidos (Florida, Texas), hasta el centro-norte de Argentina [9][5][11].

Algunas de las especies incluidas en este género, están consideradas como de importancia económica, al utilizar como plantas de alimentación, a frutos de especies cultivadas por el hombre [11]. Entre estas, se incluye a la Mosca Sudamericana de la Fruta (MSAF) (*Anastrepha fraterculus* (Wied.)), la que registra numerosas especies comerciales como hospedantes [5][8]. Los únicos países de América continental que actualmente no presentan especies del género *Anastrepha* en su territorio, son Chile y Canadá.

Sin embargo, en Chile, en 1929, se detectó el ingreso de la MSAF en el extremo norte del país limítrofe con el Perú, manteniéndose su presencia en forma recurrente hasta 1964, año en que fue erradicada definitivamente [7]. En Perú la MSAF es una especie endémica [4].

En el presente trabajo se entrega una breve reseña sobre la ocurrencia de la MSAF en Chile, desde sus primeras detecciones, hasta su erradicación del país.

2. CRONOLOGÍA DE LA MOSCA SUDAMERICANA DE LA FRUTA EN CHILE

1929   Greene [3], en su revisión del género, indica a *Anastrepha peruviana* Townsend (luego pasaría a ser sinonimia de *A fraterculus* (Wied.)), como colectada en Arica, Chile (pág. 148).

1930   Primera determinación por los Servicios de Sanidad Vegetal de Chile de *A. fraterculus* en el valle de Azapa, provincia de Arica, actual I Región del país [10].
Se dictan por parte de los Servicios de Sanidad Vegetal de Chile, una serie de decretos cuarentenarios destinados a impedir el ingreso al país de "moscas de la fruta" (Decreto N° 12 de septiembre de 1930).
Se inicia el desarrollo de una campaña erradicatoria contra la MSAF, sin lograr eliminar la plaga.

Greene [3], indica la colecta de ejemplares de *A. peruviana* (ahora sinónimo de *A. fraterculus*) en "La Maita", Arica, Chile, por parte de la expedición de Kisliuk y Cooley (pág. 148 - 149).

1940   El Servicio de Sanidad Vegetal de Chile de la época, señala como localidades con presencia de la MSAF a: valle de Azapa, Codpa, Timar y Tignamar, todas en la actual I Región. Se desarrolla una intensa campaña para erradicar la plaga de estas localidades.

1942   Alan Stone, en su obra donde revisa el género *Anastrepha*, indica a Chile como país con presencia de *A. fraterculus*, sin especificar localidades (págs.:7 y 79).

1963   Luego de permanentes campañas erradicatorias en localidades de la I Región, se definen como únicos enclaves con presencia de MSAF en Chile a: Miñe - Miñe, Cutijmalla e Iquique, todas en la actual I Región. (En este año se determina la presencia de la mosca del Mediterráneo (*Ceratits capitata* (Wied.) en el oasis de Pica y valle de Azapa, I Región).

1964   Se determinan como únicas localidades con presencia de la MSAF en Chile a: Miñe - Miñe y Cutijmalla, en la I región, donde es erradicada la plaga, luego de realizar una intensa campaña erradicatoria.

1965   Hasta la fecha (1996) No se registra la presencia de ejemplares del género *Anastrepha* en el Sistema Nacional de Detección de Moscas de la Fruta que se mantiene en forma permanente en Chile, el que incluye trampas tipo McPhail cebadas con proteína hidrolizada y el muestreo de fruta hospedera.

3. CARACTERÍSTICAS DEL ÁREA DONDE SE PRESENTÓ LA MOSCA SUDAMERICANA DE LA FRUTA EN CHILE

El extremo norte de Chile, y específicamente el área de Arica y valle de Azapa, presenta características climáticas de tipo desierto tropical marino, con una temperatura media anual de 19 °C, una máxima media del mes más cálido (febrero) de 27,4 °C, y una mínima media del mes más frío (julio y agosto) de 13,1 °C. No se registra la presencia de heladas, y las temperaturas mínimas mensuales se mantienen sobre los 10 °C. La precipitaciones anuales son de 1,1 mm, distribuidas entre julio y septiembre [6].

De acuerdo a los registros de temperatura del área ya señalados, y los antecedentes obtenidos en relación a loa umbrales térmicos mínimos de desarrollo de la MSAF, que serían 9,2 °C para huevo, 10,3 °C para larva y 10,8 °C para pupa [8], teóricamente, no existirían restricciones en este sentido para un desarrollo del insecto en la zona, siendo posible la existencia de ciclos vitales continuos a lo largo del año.

3.1. Presencia de hospederos para la MSAF

Debido a las condiciones de desierto del norte chileno, no es posible realizar actividades agrícolas sin riego, por lo que las zonas de cultivo, así como las pocas áreas verdes silvestres, se concentran en pequeñas áreas en torno a las fuentes de agua.

Sin embargo, a pesar de la reducida superficie agrícola utilizable, el extremo norte es la única zona de Chile donde es posible, dada sus condiciones climáticas, desarrollar una fruticultura con especies de tipo tropical y subtropical, como: mango, pomelo, guayabos, chirimoya, etc.

En el cuadro siguiente, se señala la situación observada en el valle de Azapa a la fecha de la detección de la MSAF, respecto a las especies frutales existentes y los períodos en que estos presentan fruta infestable (adaptado de un cuadro elaborado por Wilhelm y Olalquiaga en 1942 , y publicado en Olalquiaga y Lobos, 1993, [7]).

CUADRO 1. PERÍODO DE FRUCTIFICACIÓN DE LAS ESPECIES HOSPEDANTES DE MSAF EN EL VALLE DE AZAPA, I REGIÓN, CHILE, SEGÚN REGISTROS DE 1942.

Especie hospedante de MSAF	Período de fructificación
Guayabo (*Psidium guajaba* L.)	Mediados de marzo a mediados de octubre
Peral (*Pyrus* sp.)	Enero a abril
Pacay (*Inga feuilley* D.C.)	Mediados de marzo a mediados de julio
Chirimoyo (*Annona cherimola* Miller)	Abril a julio
Membrillo (*Cydonia oblonga* Mill.)	Mayo a junio
Ciruelo (*Spondias* sp.)	Mediados de marzo a mediados de julio
Ciruelo (*Prunus* sp.)	Mediados de enero a marzo
Duraznero (*Prunus* sp.)	Mediados de enero a marzo
Higuera (*Ficus carica* L.)	Mediados de diciembre a mayo
Chañar (*Geoffroea decorticans*)	Mediados de noviembre a mayo
Lúcumo (*Lucuma* sp.)	Mediados de noviembre a diciembre
Mango (*Mangifera indica* L.)	Febrero a marzo

De acuerdo a estos antecedentes, la MSAF disponía en el valle de Azapa, de 13 especies hospedantes principales [sin considerar a las especies registradas como dudosas por los mismos autores, como el tomate (*Lycopersicon esculentum* Miller), ají (*Capsicum* sp.), naranjo (*Citrus sinensis* (L.) Osbreck), tuna (*Opuntia ficus - indica* R. Miller), granado (*Punica granatum* L.), pepino dulce (*Solanum muricatum* Aiton.) y vid (*Vitis vinifera* L.)]. La época del año con mayor número de especies hospedantes con fruta infestable, sería el verano - otoño, y la con menos, en los meses de agosto a septiembre, donde se encontraba sólo el guayabo.

De acuerdo a estos antecedentes, la existencia de hospedantes tampoco habría sido una limitante para un desarrollo de la MSAF en el área, pudiendo aparentemente multiplicarse durante casi todo el año.

Superficie potencialmente infestable por MSAF en el extremo norte de Chile (I Región).

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Dada la condición de desierto de la I Región, las superficies agrícolas no son extensas, limitándose a zonas regadas próximas a las fuentes de agua. Se estima que actualmente existen en la I Región, bajo la cota de los 2.500 m.s.n.m., unas 30 localidades tipo oasis, donde se realiza agricultura, y existen pequeños huertos frutales, así como árboles de estas especies en los patios de las casas. Además existen dos ciudades importantes, Arica (1.600 ha) e Iquique (850 ha). De estas localidades, se detectó presencia de la MSAF sólo en 8 de ellas.

Según lo señalado en el cuadro anterior, se puede desprender que la infestación de MSAF en el área no tuvo un carácter generalizado, existiendo varios oasis donde no se detectó al insecto, a pesar de tener condiciones propicias para su establecimiento, y estar próximos a áreas infestadas de la plaga. De acuerdo con lo señalado, es posible suponer que el insecto, al momento de realizarse las primeras detecciones, se encontraba en una fase de colonización, expandiendo su área natural de establecimiento en el continente, desde, probablemente, el sur de Perú.

4. ESTRATEGIA ERRADICATORIA UTILIZADA CONTRA LA MOSCA SUDAMERICANA DE LA FRUTA EN EL NORTE DE CHILE.

Una vez confirmada la presencia de la MSAF en Chile, las autoridades de Sanidad Vegetal de la época, iniciaron en forma inmediata las actividades para la erradicación de la plaga [10]. A continuación, se señalan en forma resumida, las actividades incluidas en las campañas de erradicación de la MSAF, desarrolladas en aquellas épocas, de acuerdo a lo indicado en la literatura respectiva [10][1][2].

- Mantención de un sistema de detección en base a trampas tipo botella cebadas con vinagre al 50, 75 y 100% de concentración. La densidad de instalación utilizada fue de ca. una trampa cada 11 - 18 hectáreas.

- Definición de un período libre de hospedantes, actividad que incluyó la descarga y destrucción de fruta de huertos comerciales, árboles ornamentales y de especies silvestres (ej.:Chañar). Además se realizaron podas de árboles frutales, y cuando correspondía, destrucción de cultivos hortícolas de especies hospedantes.

- Control cultural, el que consistió en fijar períodos de cosecha de diversas frutas de los valles de las localidades infestadas con la MSAF, el que tenía el propósito de restar substrato infestable a la plaga.

- Tratamiento con cebos tóxicos para el control de adultos. Se utilizó la siguiente fórmula: azúcar 160 g, melaza 250 g, carbonato de cobre 50 g, agua hasta completar 10 litros. Esta mezcla se aplicaba a una dosis de un litro por árbol, cada tres semanas y durante cinco meses.

- Remover la capa superficial de suelo bajo la copa de los árboles frutales infestados con MSAF, lo que tenía el propósito de destruir las posibles pupas del insecto presentes en estos lugares.

- Distribución de trampas "cazadoras", de tipo botella, las que se cebaban con vinagre de vino al 20% o, con una mezcla de papilla de plátano y arseniato de plomo. Estas trampas se distribuían en alta densidad en los lugares con detección de MSAF.

- Instalación de barreras fitosanitarias temporales en las áreas de trabajo.

5. DISCUSIÓN Y CONCLUSIONES

Chile se encuentra actualmente libre de especies de moscas de la fruta del género *Anastrepha*. Sin embargo, entre 1929 y 1964, se detectó la presencia de la Mosca Sudamericana de la Fruta (MSAF) (*Anastrepha fraterculus* (Wied.) en forma localizada en algunas localidades de la I Región, erradicándose en más de oportunidad de alguna de ellas, para lograr la erradicación definitiva del país en 1964.

No se observó una dispersión y establecimiento de la MSAF en localidades al sur de la I Región de Chile, a pesar de que teóricamente, en algunas de estas áreas no presentaban limitantes de tipo climáticas o presencia de hospedantes para su establecimiento.

Dado que no se detectó en forma generalizada a la MSAF en todas las localidades con frutales hospedantes en el área infestada del norte chileno, es posible suponer que el insecto se encontraba en una fase de colonización y expansión de su limite natural de distribución.

La vía más probable de ingreso de la MSAF al extremo norte chileno, podría haber sido la migración natural del insecto desde el sur de Perú, fenómeno que posiblemente, dado el endemismo del género *Anastrepha* en dicho país, podría haberse repetido en varias oportunidades previamente (y no sólo con la MSAF). Sin embargo, sólo se ha detectado la presencia de MSAF en el período señalado, no registrándose nuevamente colectas de especies de ese género en Chile. De acuerdo con estos antecedentes, es posible suponer que las condiciones del extremo norte de Chile, si bien presentan temperaturas y una presencia de hospederos favorable para un establecimiento de la MSAF, algún factor impide que su establecimiento sea más agresivo, de modo de permitir su arraigamiento en el área. Como un ejemplo comparativo de los anterior, se puede indicar el caso de la mosca del Mediterráneo (*Ceratitis capitata* (Wied.)), la que luego de su primera detección en la misma región de Chile (1963), ha demostrado una gran agresividad dispersiva y en el establecimiento en el área (sólo se erradicó de Arica en 1995).

En Chile actualmente se mantiene en forma permanente un sistema de detección de moscas de la fruta, el que incluye un número de trampas tipo McPhail cebadas con proteína y el muestreo de fruta hospedante, destinadas a detectar el ingreso de moscas de la fruta del género *Anastrepha* en forma oportuna. Por otro lado, en las barreras fitosanitarias externas que mantiene Chile como resguardo cuarentenario, se interceptan regularmente fruta infestada con larvas de *Anastrepha* spp., lo que demuestra que el riesgo de ingreso de especies de este género a Chile se mantiene vigente.

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STATUS OF THE CARIBBEAN FRUIT FLY, *Anastrepha suspensa* (LOEW), STERILE INSECT TECHNIQUE PROGRAMME IN THE STATE OF FLORIDA, USA — NOVEMBER 1996

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Abstract

STATUS OF THE CARIBBEAN FRUIT FLY, *Anastrepha suspensa* (LOEW), STERILE INSECT TECHNIQUE PROGRAMME IN THE STATE OF FLORIDA, USA — NOVEMBER 1996.

Status of the Caribbean fruit fly, *Anastrepha suspensa*, Loew, sterile insect technique program in the state of Florida, USA-November 1996.

Application of sterilization techniques to *Anastrepha suspensa* in Florida was conducted as early as 1970 in Key West. In 1988-1990, releases of sterile flies were made in a 20 km^2 urban area in southwestern Florida adjacent to commercial citrus. With the intent to integrate a sterile insect technique system within a fly-free management program for the caribfly, additional tests are being conducted both within a major citrus production area and in an isolated urban location of the mid to lower Florida peninsula. Tests at the former site measures the synergistic effect of augmenting sterile fly releases with parasitoids, whereas the latter studies will define the efficacy of reduced numbers of sterile flies released per acre than is standard in medfly and mexfly eradication and suppression programs. Discussed here is the progress of an ongoing project to measure the benefits of SIT as it applies to caribfly export protocols.

1. INTRODUCTION

Several fruit flies in the Family Tephritidae are extremely destructive to fruit throughout the world. Not only do fruit flies cause great losses in fruit and vegetable production, but they seriously impede international trade in host commodities. Thus, they are major impediment to economic development. One of these pests, the Caribbean fruit fly, *Anastrepha suspensa* Loew was introduced into Florida on at least three occasions, becoming established in 1965 [1]. Within a few years, the caribfly had spread throughout its potential ecological range infesting more than 80 different fruit and vegetable hosts in the state.

Citrus fruit, while not a preferred host, may be successfully attacked by caribfly females, especially when the fruit are senescent. The primary impact of the caribfly has resulted from quarantine restrictions imposed on Florida by domestic and foreign export markets rather than from direct yield losses from infested citrus fruit. Currently, shipments of grapefruit, orange, lemon, lime and other citrus, plus tomato, bell pepper, lychee, mango, avocado, guava and carambola are affected by quarantine restrictions. Until 1984, most commodities were disinfected by ethylene dibromide fumigation, at which time it was banned by the US Environmental Protection Agency.

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Since then alternate methods of certifying commodities fly-free have been developed, including not only post-harvest measures, i.e. methyl bromide fumigation, cold treatment and hot water treatment, but also preharvest control strategies [2]. The latter involves certifying grapefruit and oranges from production areas if they are certified to be free of caribfly. A protocol for maintaining these areas free of caribfly similar to a program developed in the Rio Grande Valley of Texas for Mexican fruit fly, *Anastrepha ludens* Loew was incorporated in the 1982-1983 shipping season. Since 1986-1987, export of fresh citrus to Japan (4/5 bushel boxes) has equaled nearly 77 million containers.

During the development of the caribfly fly-free export protocol, future plans to expand the program to include the utilization of sterile flies was discussed and reviewed. This technique would be used to suppress caribfly populations in urban areas where the fly flourishes due to preferred host concentrations. The sterile insect technique (SIT) has been used extensively against species of fruit flies for the control or irradiation internationally such as the Mediterranean, oriental, Queensland and Mexican fruit flies, etc. [3]. Between 1970-1972 a sterile-fly release test was conducted to determine the feasibility of suppressing the caribfly in Key West, Florida [4]. This research demonstrated for the first time, using techniques as used against the aforementioned species, that suppression would be possible with the caribfly. Further studies in the late 1980s and early-to-mid 1990s with *A. suspensa* are reviewed herein.

2. MATERIALS AND METHODS

2.1. LaBelle, Florida: 1988-1990

The Florida Department of Agriculture and Consumer Services, Division of Plant Industry (DPI) in cooperation with USDA-APHIS-PPQ, conducted a three-year pilot release project between 1988-90 in an urban area of southwest Florida. The objective of the test was to measure the efficacy of sterile fly releases as they related to suppression of wild populations in high host density residential areas adjacent to commercial citrus groves. Sterile flies for use in this study were provided by the DPI Caribfly Rearing Facility staff. Procedures used, with some local modifications, were those developed for field eclosion and distribution by the PPQ Mexican Fruit Fly Rearing Facility in Texas [5]. Briefly, following tests to determine packaging, cooling and transportation methods, sterilized pupae were portioned into paper grocery bags and placed in eclosion units containing food and water. At sexual maturity the sterile flies were released, by roving release over a pre-designated treatment area at a target over-flooding ratio of 500:1 (sterile:wild). To monitor the reduction of wild flies over the release area, McPhail traps were set accordingly within both the treated and non-treated area. Traps were inspected weekly for the presence of wild and/or sterile flies.

To insure that the product in the field was a characteristic composite of that in the production facility, quality control tests were both observed at the rearing lab (i.e. pre and post irradiation) and at the field station (i.e. to measure packaging/transportation effect, if any). Each shipment was monitored either and/or for the following parameters: emergence, flight ability, sex ratio, sterility, and mating propensity. These measurements follow closely those developed for use in Medfly to evaluate production, process and product quality control [6]. (These procedures are presently being updated to include various other Tephritidae species of program significance.) If any discrepancies were noted in the quality of the product received, or shipping problems were apparent, the administrator of the rearing facility was contacted and corrections to the problem(s) were addressed.

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2.2. St. Lucie County, Florida: 1995

In conjunction with the cooperative caribfly parasitoid augmentation project conducted in St. Lucie County (the Ft. Pierce vicinity), an average of 1,444,100 sterile flies per week were released over ca. 17 square-mile area in a period of 30 weeks. Sterile fly liberations were in the general vicinity of the 47 parasitoid release sites *(Diachasmimorpha longicaudata:* Family Braconidae) on a weekly basis. Fly releases were initiated the week ending May 5, 1995 and continued thru the week ending November 24, 1995. Unlike the release methodology used in the LaBelle studies, sterile flies were allowed to light from the eclosion unit at specific sites. Fifty traps were used to monitor the ratio of sterile to wild flies recovered. The period in which the steriles were released generally coincides with the time commercial citrus is not being harvested. This was to avoid possible initial confusion between test flies (marked with a fluorescent dye) and wild flies in nearby export protocol traps. As for the LaBelle study, sterilized pupae were received from the DPI production facility in Gainesville. Likewise, quality of shipments was measured.

2.3. Clewiston, Florida: 1996-Present

Tests were initiated in June of 1996 in Clewiston (located on the southeast shoreline of Lake Okeechobee) to determine if sterile flies can be efficacious (suppress caribfly densities below Probit-9 levels) at release rates of 200 per acre. Due to lab production capabilities and the area ultimately targeted for release, i.e. the major citrus export areas of Indian River, it becomes necessary to distribute flies as thinly as possible without bringing about deleterious consequences. Steriles are presently being released over an urban area of ca. 5.5-6.0 sq mi., at weekly intervals, at 40 sites from eclosion units. Eighty (80) McPhail traps are serviced weekly. The sterile fly source and quality control procedures used for this test parallel those described for St. Lucie.

2.4. Ground-release sterile fly equipment

A prototype truck-mounted refrigerated insect release machine is presently being evaluated as an alternative to static/roving release techniques. This is the same chilled fly/auger dispensing system used to aerially release Mediterranean/Mexican fruit fly in APHIS-PPQ Action Programs. Rather than ejecting steriles through a shoot connected to a hole in a bottom of an airplane, flies are directed to the side of the road (adjacent to residential host material) through a tube extending from the release unit assembly. Plans are to field test this unit in Clewiston by comparing flies trapped between this method and the standard "hand-delivery" procedure.

2.5. Sterile/wild caribfly ID Manual

Prior to instituting area-wide aerial release of caribfly, we – the citrus producers/exporters and DPI – must be assured that flies trapped in or around a grove can be properly and expediently identified as either sterile or wild. Therefore, the development of a technical identification handbook illustrating irradiation damage to male/female caribflies - "0"-14 days old is nearing completion. Similarly, the same sequence of color prints for unirradiated flies will be included in the manual. This tool should enhance the reliability in trapped fly determination.

TABLE I. RELEASE, RECOVERY, AND STERILE-TO-WILD RATIO OF CARIBBEAN FRUIT FLIES IN LABELLE, FLORIDA, 1988.

1988	NUMBER OF FLIES RELEASED (MILLION)	% RECOVERY	RATIO TRAPPED: STERILE TO WILD
JAN	0.39	0.09	N/A
FEB	0.84	0.18	118
MAR	0.81	0.39	286
APR	0.39	1.00	48
MAY	0.79	0.15	17
JUN	0.15	0.74	27

TABLE II. RELEASE, RECOVERY, AND STERILE-TO-WILD RATIO OF CARIBBEAN FRUIT FLIES IN LABELLE, FLORIDA, 1989.

1989	NUMBER OF FLIES RELEASED (MILLION)	% RECOVERY	RATIO TRAPPED: STERILE TO WILD
FEB	1.25	0.11	18
MAR	1.25	0.24	172
APR	2.26	0.15	524
MAY	1.53	0.08	234
JUN	2.26	0.07	1,327

TABLE III. RELEASE, RECOVERY, AND STERILE-TO-WILD RATIO OF CARIBBEAN FRUIT FLIES IN LABELLE, FLORIDA, SEPTEMBER 1989-MAY 1990.

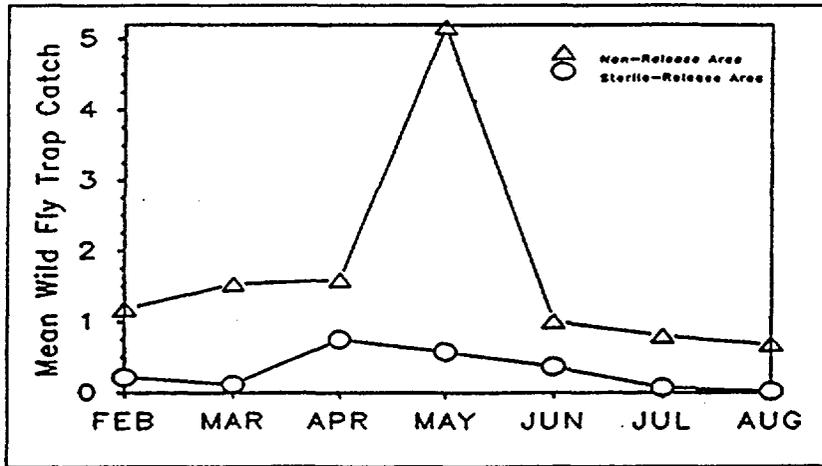| 1989-1990	NUMBER OF FLIES RELEASED (MILLION)	% RECOVERY	RATIO TRAPPED: STERILE TO WILD
SEP	2.82	0.02	N/A
OCT	1.38	0.40	185
NOV	1.84	0.12	580
DEC	2.18	0.12	542
JAN	1.97	0.27	2,334
FEB	1.50	0.29	1,117
MAR	3.64	0.12	2,725
APR	3.25	0.36	1,893
MAY	3.26	0.20	680

Figure I. 1988 MEAN WILD FLY TRAP CATCH (MONTHLY) IN RELEASE AND NON-RELEASE AREA, LABELLE, FLORIDA.
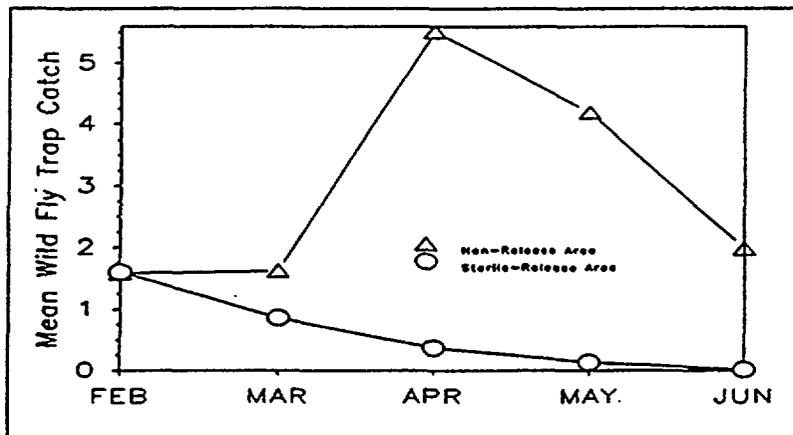

Figure II. 1989 MEAN WILD FLY TRAP CATCH (MONTHLY) IN RELEASE AND NON-RELEASE AREAS, LABELLE, FLORIDA.
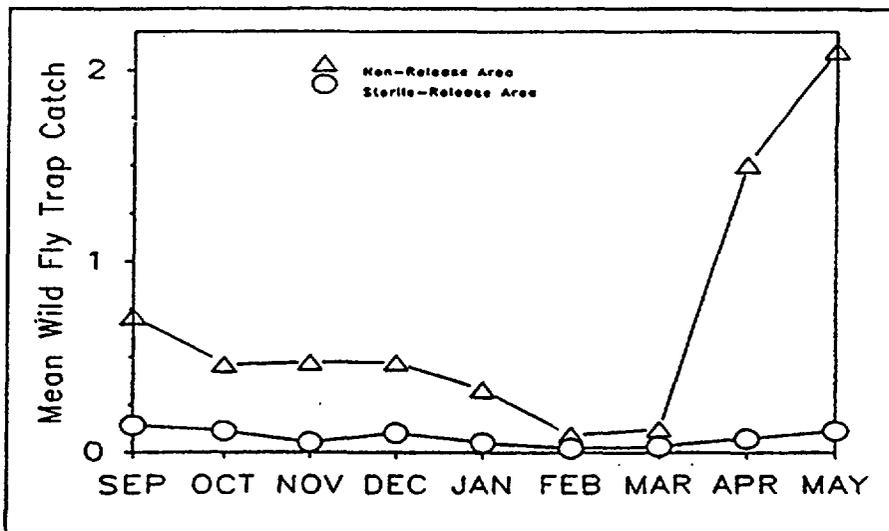

Figure III. 1989-90 MEAN WILD FLY TRAP CATCH (MONTHLY) IN RELEASE AND NON-RELEASE AREAS, LABELLE, FLORIDA.

3. RESULTS AND DISCUSSION

3.1. LaBelle, Florida

Results from this test have previously been reported [7] but will be discussed here briefly. The reduction of the caribfly in the urban area of LaBelle as measured for the three test periods (1988, 1989 and September 1989 thru May 1990) is illustrated in Tables I-III and Figures 1-3. Overall, as sterile-to-wild fly ratios improved from test period to test period, the caribfly population decreased substantially. This is apparent specifically in the 1989-90 study where the quality of the flies and the numbers released (with the exception of one of the nine months steriles were liberated) were within project specifications. We were able to determine, as observed in sterile mexfly releases in the Rio Grande Valley, that to successfully suppress caribfly, sterile flies should be directed at low population densities (i.e. during a period of time in which host fruits are generally absent).

TABLE IV. SEASONAL RESULTS FOR A CARIBBEAN FRUIT FLY, ANASTREPHA SUSPENSA, LOEW STERILE INSECT TECHNIQUE GROUND RELEASE PROGRAM- ST. LUCIE COUNTY, FLORIDA, USA 1995[a]

Week Ending	Number Sterile Flies Released	No. Traps	Number Sterile Flies Recovered	Number Wild Flies Recovered	Number Wild Flies Per Trap	Percent Recovery Sterile Flies	Ratio Trapped Sterile:Wild
05/05/95	204,250	50	0	71	1.42	0.0000%	0.00
05/12/95	985,320	50	0	107	2.14	0.0000%	0.00
05/19/95	930,900	50	2	139	2.78	0.0002%	0.01
05/26/95	1,120,080	50	12	755	15.1	0.0011%	0.02
06/02/95	947,760	50	21	464	9.28	0.0022%	0.05
06/09/95	1,023,000	50	8	171	3.42	0.0008%	0.05
06/16/95	1,068,000	50	28	239	4.78	0.0026%	0.12
06/23/95	1,110,000	50	19	123	2.46	0.0017%	0.15
06/30/95	1,116,000	50	10	144	2.88	0.0009%	0.07
07/07/95	1,122,000	50	288	159	3.18	0.0257%	1.81
07/14/95	1,244,880	50	41	254	5.08	0.0033%	0.16
07/21/95	1,227,600	50	61	295	5.90	0.0050%	0.21
07/28/95	1,240,800	50	50	248	4.96	0.0040%	0.20
08/04/95	2,073,600	50	104	174	3.48	0.0050%	0.60
08/11/95	928,880	50	21	22	0.44	0.0023%	0.95
08/18/95	1,953,840	50	9	79	1.58	0.0005%	0.11
08/25/95	1,976,400	50	17	155	3.10	0.0009%	0.11
09/01/95	982,800	50	28	97	1.94	0.0028%	0.29
09/08/95	1,878,000	50	12	29	0.58	0.0006%	0.41
09/15/95	1,821,120	50	14	28	0.56	0.0008%	0.50
09/22/95	1,910,640	50	38	55	1.10	0.0020%	0.69
09/29/95	1,869,600	50	20	22	0.44	0.0011%	0.91
10/06/95	1,965,600	50	8	21	0.42	0.0004%	0.38
10/13/95	972,000	50	139	8	0.16	0.0143%	17.38
10/20/95	2,064,480	50	12	7	0.14	0.0006%	1.71
10/27/95	1,954,800	50	0	6	0.12	0.0000%	0.00
11/03/95	1,900,800	50	14	10	0.20	0.0007%	1.40
11/10/95	1,838,960	50	10	4	0.08	0.0005%	2.50
11/17/95	1,836,000	50	23	3	0.06	0.0013%	7.67
11/24/95	1,954,800	50	40	22	0.44	0.0020%	1.82
12/01/95	0	50	13	14	0.28	ERR	0.93
12/08/95	0	50	16	8	0.16	ERR	2.00
12/15/95	0	50	67	23	0.46	ERR	2.91
12/22/95	0	50	43	24	0.48	ERR	1.79
MEANS	1,444,097					0.0027%	1.34

[a] A cooperative program conducted by FDACS, Division of Plant Industry and USDA-APHIS-PPQ

TABLE V. PRELIMINARY RESULTS OF A CARIBBEAN FRUIT FLY, ANASTREPHA SUSPENSA, LOEW STERILE INSECT TECHNIQUE GROUND RELEASE PROGRAM- CLEWISTON, FLORIDA, USA 1996 [a]

Week Ending	No. Traps	No. Sterile Flies Released	No. Sterile Flies Recovered	No. Wild Flies Recovered	No. Wild Flies Per Trap	Percent Recovery Sterile Flies	Ratio Trapped Sterile:Wild(1)
06/09/96	71	634,800	274	2303	32.4	0.04%	0.12
06/16/96	76	608,760	509	1054	13.9	0.08%	0.48
06/23/96	82	607,200	534	846	10.3	0.09%	0.63
06/30/96	83	684,000	543	877	10.6	0.08%	0.62
07/07/96	79	655,200	611	306	3.9	0.09%	2.00
07/14/96	76	684,000	397	402	5.3	0.06%	0.99
07/21/96	75	676,800	272	272	3.6	0.04%	1.00
07/28/96	80	752,400	1461	741	9.3	0.19%	1.97
08/04/96	76	662,400	868	755	9.9	0.13%	1.15
08/11/96	78	789,600	912	935	12.0	0.12%	0.98
08/18/96	75	829,440	969	1039	13.9	0.12%	0.93
08/25/96	75	834,720	2206	1144	15.3	0.26%	1.93
09/01/96	75	861,360	1104	837	11.2	0.13%	1.32
MEANS		713,898				0.11%	1.09

[a]) A cooperative program conducted by FDACS, Division of Plant Industry and USDA-APHIS-PPQ

3.2. St. Lucie County, Florida

Results for the 30-week sterile caribfly program is listed in Table IV. Means indicate that the ratio of trapped sterile flies to wild flies is significantly lower than preferred (i.e. "season" three in LaBelle). This is considerably lower than 500 steriles per acre (standard). However, there may have been (although non-qualitative) a measure of efficacy during the period of time which steriles were released along with parasitoids. This decrease in caribfly as measured both by trap catch and the reduction in fruit infestation, was orally presented at the annual Florida Entomological Society meetings in August 1996 [8]. (Data for a study conducted in Key Biscayne in the early 90s utilizing both steriles and *D. longicaudata* have not been analyzed to date, as a question to the reliability of the information collected arose-J. Sivinski, personal communication.) Future activity in the project is dependent upon the tests presently being conducted in Clewiston.

3.3. Clewiston, Florida

Results through the week ending September 1, 1996 for the Clewiston study are found in Table V. Means indicate that the ratio of trapped sterile flies to wild flies 13 weeks into the project perhaps is not sufficient. (Note: In this test, expected trap attractant ratio should be in the range of 40 percent less than if 500 sterile flies were being released per acre.) Sterile fly

CARIBBEAN FRUIT FLY QUALTIY ASSURANCE REPORT
STERILE INSECT TECHNIQUE RELEASE PROJECT

JULY-SEPT 1996

SHIP #	PUPAE DATE	PUPAL WT/10 (mg)		% EMERGED (FLIGHT TUBES) COLONY n=5		IRRADIATED n=5		RELEASE n=4		% MALES (FROM SEX RATIO) COLONY n=5		IRRADIATED n=5		RELEASE n=4		% ABSOLUTE FLIERS (FLIERS/PUPAE) COLONY n=5		IRRADIATED n=5		RELEASE n=4		MATING INDEX COLONY n=5		IRRADIATE n=5	
		AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.
JUL SHIPM'T																									
6	06/19	10.8	0.2	94%	0%	93%	0%	97%	2%	59%	2%	62%	1%	59%	2%	90%	1%	86%	1%	91%	2%	85	5	84	6
7	06/26	11.9	0.2	93%	2%	90%	1%	94%	1%	44%	3%	47%	2%	39%	2%	91%	1%	87%	1%	87%	2%	85	5	84	6
8	07/03	11.2	0.2	86%	4%	89%	2%	95%	1%	52%	2%	48%	3%	51%	3%	66%	6%	73%	1%	87%	2%	80	5	76	10
9	07/10	11.3	0.1	86%	1%	88%	2%	92%	1%	52%	2%	53%	1%	53%	5%	72%	2%	72%	2%	79%	2%	87	2	83	6
10	07/17	11.8	0.2	85%	3%	88%	2%	94%	1%	53%	1%	50%	1%	58%	2%	72%	2%	74%	3%	80%	2%	91	4	88	6
AUG SHIPM'T																									
11	07/24	11.0	0.2	89%	1%	82%	2%	96%	1%	58%	1%	46%	1%	56%	2%	76%	3%	68%	2%	86%	1%	79	3	80	3
12	07/31	11.4	0.2	92%	1%	94%	1%	94%	2%	60%	1%	66%	2%	63%	2%	82%	1%	78%	1%	76%	2%	81	3	70	6
13	08/07	11.9	0.2	91%	2%	82%	3%	97%	1%	46%	1%	53%	1%	51%	4%	80%	1%	66%	3%	81%	1%	90	4	45	25
14	08/14	11.6	0.2	92%	1%	89%	1%	94%	0%	62%	2%	53%	1%	62%	3%	87%	1%	78%	1%	88%	1%	87	5	80	2
SEP SHIPM'T																									
15	08/21	11.1	0.2	95%	1%	87%	1%	95%	1%	65%	1%	64%	1%	67%	4%	88%	2%	75%	2%	84%	2%	81	7	86	7
16	08/28	11.1	0.3	85%	2%	83%	1%	92%	2%	66%	1%	65%	1%	60%	3%	77%	3%	74%	1%	79%	2%	87	4	83	7
17	09/04	11.1	0.2	92%	1%	91%	2%	96%	1%	60%	3%	66%	2%	62%	1%	88%	1%	84%	2%	79%	1%	89	4	82	8
18	09/11	10.8	0.2	93%	2%	93%	1%	98%	1%	56%	1%	58%	1%	60%	1%	88%	2%	83%	1%	88%	2%	86	5	89	7
19	09/18	11.3	0.3	91%	1%	92%	1%	99%	1%	73%	1%	75%	2%	68%	4%	83%	2%	80%	2%	90%	0%	92	3	89	3
QUARTER AVERAGE		11.3		91%		89%		95%		58%		57%		58%		81%		77%		84%		86		80	
STD. DEV.		0.4		3%		4%		2%		8%		9%		7%		7%		6%		5%		4		11	

PUPAE DATE COMMENTS:

06/19 Reported data from Release Center for % fliers computed with n=3, instead of n=4

07/10 The reduction in % Fliers between Colony and Irradiated/Release suggest possible irradiation damage.

07/31 There was a 20% increase in male non-fliers in irradiated & release ctr. samples and 11pt drop in MPI suggesting irradiation damage.

08/07 MPI of irrad reduced 50% relative to Colony males indicates irradiation damage believed due to premature irradiation

The elevated numbers reported from the release center are most likely the result of the differences in the sampling techniques employed. A mechanical sampling device has been developed and is being tested. A mechanical sampler will be installed at the Release Center asap to assure equivalent sampling techniques are used at all stages of the SIT process.

recovery during this reporting period certainly was limited by high air temperatures. A residue of wild caribflies may have also skewed the sterile/wild ratio in favor of the wild fly due to the accumulation of caribfly progeny building-up over the spring and early summer. Recalling that releases targeted for the fall and winter season, when fly densities are at the lowest (due again to the lack of hosts) are preferable, information gathered by this period next season may perhaps indicate significant suppression. (Also recall that for a short period of time in LaBelle-1988, suppression was noted at the 200 sterile fly level.)

3.4. Ground release equipment

This unit was first demonstrated in the Lower Rio Grande Valley of Texas in cooperation with PPQ Mexican Fruit Fly Rearing Facility personnel, the PPQ-Aircraft and Equipment Operations staff and representatives of the California and Florida Departments of Agriculture. Following the demonstration, minor changes/additions to the unit were recommended. With the corrections made, the unit was field tested again (in a residential area of Mission) and data collected from traps were less than encouraging. Arrangements are being made to have the unit tested in Florida at the beginning of the calendar year.

3.5. Identification manual

All that remains to accomplish is completion of a series of 0-14 day old irradiated male caribfly photographs and line-drawings of morphological characteristics of the male and female (unirradiated) reproductive systems (including spermatheca). The formulation for the cytological stain used in preparations of caribfly testes and ovaries for slide presentation will also be included. This manual, more complete in scope, follows one (unpublished) hastily organized in the early 1980s for use in the Miami, Florida Medfly Emergency Eradication Program.

ACKNOWLEDGEMENTS

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POTENTIAL USE OF THE STERILE INSECT TECHNIQUE
AGAINST THE SOUTH AMERICAN FRUIT FLY

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Abstract

POTENTIAL USE OF THE STERILE INSECT TECHNIQUE AGAINST THE SOUTH AMERICAN FRUIT FLY.

The Latin American countries have a strong interest in increasing fruit production and quality to facilitate commercialization within and outside the region. Various fruit fly control programmes in South America and their objectives and benefits are described here. Specific priorities to improve fruit fly control and eradication technologies include strengthening of quarantine, development of pre- and post-phytosanitary measures, and harmonization of the most effective and advanced technical procedures/methodologies to control fruit flies. A subregional strategy to control fruit flies in South America would promote technical co-operation among the South American countries and strengthen the activities of less advanced fruit fly programmes. Effective use can be made of local/regional infrastructure, expertise, sterile fly production and human/technical resources. In Argentina, advanced technology related to the use of medfly genetic sexing strains for SIT programmes has been successfully introduced. Joint efforts between technicians and scientists would contribute to developing new technology to control important pests in South America.

1. INTRODUCTION

Over the last four years, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has been directly involved in the development of programmes and technology transfer on the use of the Sterile Insect Technique (SIT) in South America. Two national-IAEA Technical Cooperation Projects with the aim of eradicating the Mediterranean fruit fly (medfly) are being successfully implemented in Argentina and Chile. Other South American countries suffering similar fruit fly problems, have expressed interest in also obtaining technical support in the environment-friendly control of these pests.

The medfly and other fruit flies of economic and quarantine importance are causing important economic losses to the countries of the region. These losses can be divided into two categories: a) those caused by the direct attack of the pest to fruits and vegetables in the field (from 3 to 50%) in spite of insecticide applications, and b) the indirect damage to the fruit industry by preventing fresh fruit and vegetables exports to fruit fly free countries (considerable losses, not calculated). Other associated problems in fruit production are the excessive use of insecticides (in most cases to control fruit flies), the problem of pesticide residues on fruits and vegetables and the possible selection for fruit flies resistant to insecticides.

Fruit flies are widely distributed in South America. The Mediterranean fruit fly is an exotic pest for this subregion, having been introduced only this century. It is considered the most important threat to fruit production and fruit industry development. The importance of

MAP 1: Hypothetical distribution of the important fruit flies in South America

FRUIT FLY FREE ZONES

ONLY MEDFLY

MEDFLY AND THE SOUTHAMERICAN FRUIT FLY

MORE THAN THREE SPECIES OF FRUIT FLIES OF IMPORTANCE
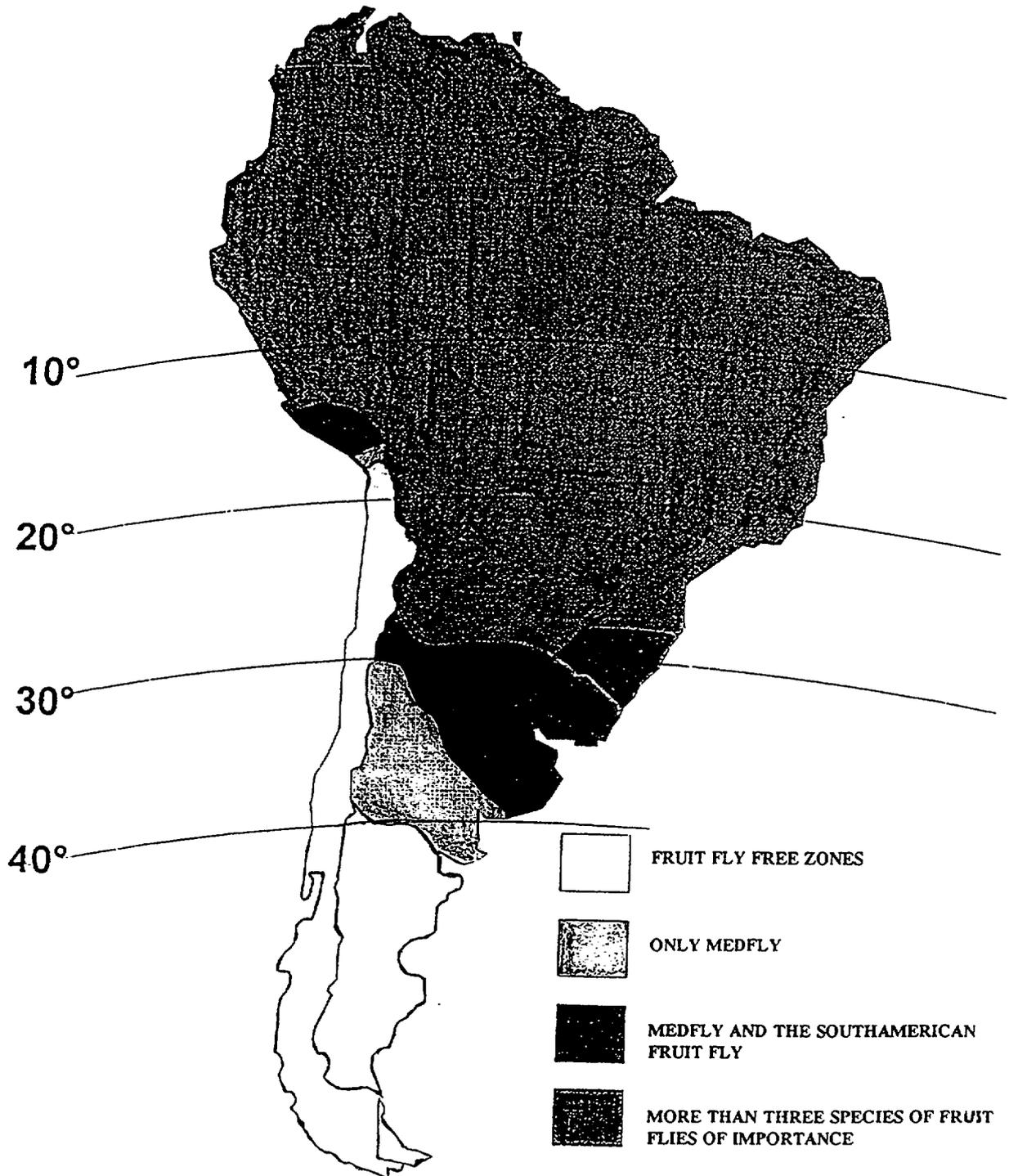
medfly is due to its great reproductive potential, wide range of fruit hosts and its great adaptation capacity to extreme weather conditions. In South America, medfly is established even at 40° southern latitude in Patagonia, Argentina. Most of the other endemic fruit flies are found north of 32° southern latitude (some exceptions can be found in the southernmost valleys of Perú and in other specific or isolated valleys of the countries). This situation allows the technical feasibility to maintain a fruit fly free status in Chile and the creation of important fruit fly free zones in Argentina and Perú by eradicating medfly using the SIT (see Map 1).

Chile has succesfully eradicated medfly several times, maintaining a fruit fly free zone for the most important fruit production areas of the country located from 20° southern latitude to the south. This phytosanitary status has been decisive in the strong development of the Chilean fruit export industry, with a current value of more than US$ 1 billion per year. Recently, using SIT, Chile eradicated the pest from the geographically isolated and northernmost part of the country, the Arica valley, at the border with Peru. Then, Chile was offically declared a medfly free country in December, 1995, and the eradication efforts are being moved to the southernmost valleys in Perú, the Tacna and Moquegua valleys.

The use of SIT has been demonstrated to be an effective and sustainable way to control the pest, generating great interest in neighboring countries, which are very interested in obtaining this technology to deal with their fruit fly pests.

Argentina is currently conducting an important programme using the SIT to eradicate medfly from more than 700,000 ha of cultivated land in the isolated fruit-producing valleys of the Patagonia region as well as in Mendoza and San Juan provinces.

Perú has embarked on an ambitious fruit fly control and eradication project mainly oriented to the coastal fruit producing valleys of the country. The government is investing important resources to build 3 fruit fly and parasitoid mass rearing facilities, one in the north, the refurbishment of the "La Molina" mass rearing facility in Lima and another in the south of the country. In addition, Perú and Chile have established an active bilateral agreement to eradicate medfly and the South American fruit fly from the southernmost valleys of Perú.

The rest of the countries in South America are interested in increasing fruit production and exports, because the fruit fly problem is one of the most important limitations to be overcome.

2. ADVANTAGES OF ESTABLISHING A SUBREGIONAL STRATEGY TO CONTROL THE FRUIT FLIES IN SOUTH AMERICA.

Many countries in South America have expressed a strong interest in developing up-dated technology to control and to eradicate fruit flies of economic and/or quarantine importance. Among the objectives that these countries would like to achieve are:

- To increase quality and production of fruits and vegetables in order to facilitate commercialization within and outside the region.

- To protect the environment by reducing the use of pesticides by promoting environment-friendly pest control strategies in fruit and vegetable production .

- To promote technical cooperation among the countries to take advantage of the infrastructure and the human/technical resources presently available in the subregion to strenghten the activities of less advanced fruit fly programmes.

Among the specific priorities to be addressed to improve fruit fly control and eradication technologies in the countries are:

- The harmonization of the most effective and advanced technical procedures/methodologies to control fruit flies, especially the medfly and the South American fruit fly,

- To strengthen knowledge on integrated fruit fly control techniques and the use of the Sterile Insect Technique to control and eradicate medfly and other fruit flies of economic and quarantine importance,

- Use of the present infrastructure in Argentina and Chile as specialized SIT training centers in the subregion.

- Development of the necessary pre- and post-harvest phytosanitary measures, including fruit fly quarantine treatments for fresh fruit and vegetables, to overcome quarantine restrictions during the export process.

- Strengthening of the national quarantine infrastructure to reduce the risks of introduction of exotic pests and to avoid the movement of established pests inside the subregion to protect the fruit fly eradication programmes.

In view of the above mentioned points, a subregional strategy is needed to promote activities for the establishment of an effective technical coordination among the interested countries. The countries can already take advantage of the existing infrastructure, the sterile fly production and the experience on medfly SIT area-wide control and eradication programmes and plant quarantine systems presently available and succesfully developed in Chile and Argentina.

The current National Fruit Fly Programmes in Chile, Argentina, Perú, Ecuador, Colombia, Uruguay and Brasil, managed under the National Plant Protection Organizations of the Ministries of Agriculture, would be the framework within which to develop a subregional project strategy. The countries would continue assuming the responsibility for their own national fruit fly programmes with their specific objectives. However, the technical cooperation and active interaction/communication among the different National Fruit Fly Programme Coordinators and/or the National Plant Protection counterparts is very low or, in many cases, does not exist. Efforts should be made to encourage technical cooperation among neighboring countries in the subregion with the assistance of the Regional or International Organizations such as COSAVE, IICA, FAO, IAEA, USDA, GTZ, EU, etc. in order to promote the following activities and policies:

- Assemble the National Fruit Fly Coordinators of the interested countries in a first meeting to review the programmes and find points of interest/collaboration/training, etc. and establish a chronogram for periodic meetings (every 3 months, for example).

- Support the organization and execution of subregional technical meetings and training courses.

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- Request economic support for research and specific technical advice through regional and international experts to develop the SIT in *Anastrepha fraterculus*.

- Provide specialized training to the key professional staff of the national fruit fly programmes in the countries through the provision of fellowships and scientific visits in and outside the subregion.

- Promote the establishment of specific agreements for the provision of sterile flies and technical assistance among the countries.

- Provide specific equipment and materials not available in the subregion to strengthen the field and laboratory fruit fly programmes Prepare and distribute an inventory of experts on fruit flies available in the subregion, Latin America and the U.S.

The interaction of the existing valuable human resources and the use of physical and technical resources now available in the subregion will be of great acceptance and will result in important benefits to the on-going and future fruit fly programmes in the South American countries.

3. PHYTOSANITARY CONCEPTS TO BE CONSIDERED

The countries interested in improving productivity and increasing exports of their fresh fruit production should establish concrete activities/programmes on an area-wide pest management basis. This concept should be very well understood by Plant Protection leaders and fruit growers' organizations. Policies oriented to promote the fruit growers' organization are indispensable in the process to apply phytosanitary programmes on an area-wide basis.

An indispensable and important action to secure these intentions is the creation of "Fruit Fly Free Areas" (FFFA) and "Fruit Fly Low Prevalence Areas" (FFLPA). The establishment of these technical and protocolar concepts will be a very important step in the process to eliminate the current quarantine restrictions imposed to the fruit and vegetable produce of the countries by fly free countries/markets.

FFFA and FFLPA that are officially and internationally recognized are the phytosanitary instruments approved by the International Plant Protection Convention (IPPC) and adopted or in course of adoption by the Regional Plant Protection Organizations (RPPOs) and the National Plant Protection Agencies (NPPAs) to allow free commercialization of healthy commodities to competitive markets. In addition, and as a result of the adoption of the GATT agreement and the latest developments within the regional common market MERCOSUR, these phytosanitary instruments are becoming increasingly important for trading agricultural produce.

The participating countries will benefit significantly by increasing fresh fruit production and exports through the establishment of such phytosanitary measures. A coordinated technical effort between the countries will make effective use of the local/regional infrastructure, expertise, sterile fly production and the wide experience in fruit fly control and eradication technology now available in Chile and Argentina. Moreover, Chile could provide advice in plant quarantine technology and postharvest treatments of fresh fruit and vegetables.

4. FUTURE OF FRUIT FLY SIT PROGRAMMES IN THE SUBREGION:

Presently, the use of the SIT in South America is oriented only to the eradication of medfly, in fruit-producing zones where medfly is the only fruit fly of economic and quarantine importance. These zones are located only in the southern part of Argentina and Perú and are feasible candidates to be FFFA. There are other important fruit-producing areas in Argentina, Uruguay, Brasil and Perú, where Medfly coexists with the South American fruit fly (SAFF)(*Anastrepha fraterculus*), that are candidates to create FFFA but only by developing the SIT to eradicate the SAFF. An immediate alternative to succesful control of these fruit flies in these zones is the creation of FFLPA. This strategy will be valid if the costs of the fruit fly control programmes are lower than those of traditional control programmes through the use of insecticides presently applied by the fruit growers. For the long term, SIT control programmes against Medfly and SAFF will allow an important reduction of insecticide use, resulting not only in a more effective control of these species, but with benefits to the environment and the quality of the fruits and vegetables.

Medfly SIT eradication programmes to create FFFA are feasible when the following specific conditions are accomplished: geographical isolation, minimum road communications to the zone, high values of the fruit produced and the active participation of the fruit growers and governments to establish the necessary strict quarantine regulations. These conditions together are not easy to find in most of the fruit-producing zones of the countries. So, the future of Medfly SIT eradication programmes are limited to the zones in which these factors are met. Joint SAFF and medfly SIT eradication programmes are not possible to establish at this time, due mainly to the lack of SIT technology to eradicate the SAFF. If a strong research programme to develop the SIT for SAFF is now established in South America, the technology could be available for area-wide application in as little as two to three years.

Control programmes to create FFLPA rather than FFFA are, at this time, technically and economically feasible for implementation in many fruit-producing areas of the countries. The FFLPA concept should be developed and well understood by Plant Protection leaders in the subregion in order to establish the conditions to succesfully apply the phytosanitary regulations, field activities and participation of fruit growers and governments that are required. By developing and strengthening this concept, the fruit and vegetable production of a determined zone/region or country, can be exported to the present restricted markets through the bilateral negotiations between the countries involved. These bilateral negotiations must result in the establishment of a mutually agreeable protocol which normally states:

- The phytosanitary and quarantine measures (quarantine treatments) that should be accomplished by the exporting country,

- The participation of the growers/organizations/authorities involved (fruit growers, packing enterprises, plant quarantine inspectors of both countries, etc),

- The field activities to maintain the status of FFLPA

- The actions to be taken in case of pest outbreaks in the field, pest detection during packing, or in the shipments to the international market.

5. CURRENT STERILE FLY PRODUCTION IN SOUTH AMERICA

Both, Argentina and Chile have medfly mass rearing and sterilization facilities in full operation with a present production capacity of 280 million sterile flies per week. Moreover, with the improvements currently being made by Peruvians to their facility at "La Molina," the production potential will be increased to 340 million sterile flies per week. The present production of sterile flies is being released in the current medfly eradication programmes in Argentina, Chile and Perú. Technicians and scientists from these medfly rearing facilities already have the experience to provide technical assistance to neighboring countries. In addition, advanced technology related to the use of medfly genetic sexing strains for SIT programmes has been succesfully introduced in Argentina. This new technology, which consists of producing and releasing male-only sterile medflies, is being used with high expectations of success in Mendoza, Argentina.

Chile is in the process of introducing this technology and in 1997 will be producing and releasing male-only sterile medflies using a genetic sexing strain.

6. STERILE SAFF PRODUCTION PLANS

Presently, Perú is the only country in South America with an ambitious plan to eradicate fruit flies of economic and quarantine importance from the fruit-producing valleys of the Peruvian Pacific coast. Fruit fly eradication includes the Medfly, SAFF and *A. obliqua*. In Argentina, important fruit producing zones are technically and economically feasible candidates to apply SIT programmes for medfly in conjunction with SAFF. However, the research efforts to develop the SIT for SAFF are very isolated, and there is not a visible policy in the government to support this activity. For this reason, it is very important to promote regional events among technicians and scientists related to fruit fly programmes in order to join efforts and convince the high level agricultural authorities and plant protection leaders of the importance of developing new technology to control important pests such as the South American fruit fly.

7. CURRENT FRUIT FLY PROGRAMMES IN THE SUBREGION:

Chile, the only country in South America internationally recognized as a fruit fly free country, has developed an effective and profitable phytosanitary programme to maintain this status. Medfly was finally eradicated from the small and isolated area of Arica, in the northernmost part of the country, at the border with Perú. Here, the Chilean Agricultural Service (SAG), through the National Plant Protection Institute with the support of the IAEA succesfully achieved the eradication of this pest using the Sterile Insect Technique. This achievement will allow the Chilean fruit industry to proceed with fruit and vegetable shipments to Asian markets. The government and the private sector provided strong support to the programme to maintain the fruit fly free status and, in consequence, there is a growing fresh fruit export industry.

Currently, Chilean Agriculture authorities are looking to their neighbours to help them protect their fruit industries with the aim of expanding the control of fruit flies and thereby eventually increasing the fly free buffer area around Chile. With Perú, a phytosanitary agreement was established in 1991 to control the medfly in the Peruvian valley of Tacna, the area bordering Chile. Under this bilateral agreement, Chile is providing to Perú in 1995, 20 million sterile flies per week from the new medfly mass rearing and sterilization facility in

Arica as well as operational funds (approx. US$ 350,000 per year) to eradicate the medfly from the southern valleys in Perú. With Argentina, the establishment of bilateral agreements to support medfly eradication from the neighbouring provinces of San Juan, Mendoza and Patagonia are in progress, including technical support given to upgrade Argentinean fruit fly quarantine procedures. Chile is requesting from IAEA the continuation of the technical assistance to complete medfly eradication in its territory and to strenghten the bilateral medfly programme with Perú.

Argentina, with a high potential to export large volumes of fresh fruit, embarked in 1993, with the support of IAEA, on an ambitious National Fruit Fly Programme. Concrete medfly eradication activities are in progress in 500,000 ha of the province of Mendoza. This programme will be expanded in the near future with the support of FAO to the provinces in Patagonia and San Juan province. Expansion to the citrus area in the eastern part of the country, at the border with Uruguay, is also under consideration.

From 1990 to 1995, more than US$ 25 million have been invested in the establishment and operation of 25 internal quarantine stations, the infrastructure and operation to produce and sterilize more than 200 million insects per week, the training of personnel and the field operations to control the pest over half a million hectares. During 1995, the use of genetic sexing strains to release male-only sterile medflies will significantly improve the SIT and will reduce the operational costs of the eradication programmes. This male-only technology can then be transferred into other facilities in the subregion, including the Arica plant in Chile and the La Molina plant in Peru.

Argentina and Chile would greatly benefit by closer coordination of efforts against fruit flies. On the one hand, Argentina requires Chilean recognition of the fruit fly free areas of Mendoza, San Juan and Patagonia in order to be able to move fruits and vegetables through Chilean territory and to use Chilean ports on the Pacific Ocean. On the other hand, Chile is very interested in the success of the medfly eradication programmes in Argentina because they will allow movement of the quarantine barrier furhter east into Argentina, thereby providing more protection and drastically reducing the incidence of costly annual pest introductions into Chile.

Argentina is requesting from IAEA the continuation of the technical assistance to finalize eradication of medfly from Patagonia and Cuyo provinces and to expand activities to the important Litoral citrus-producing areas.

Perú is up-grading its fruit fly programme. Plant protection officials are interested in acquiring a new irradiator for their mass rearing facility at La Molina, as well as in introducing new rearing technologies. In addition to the medfly control activities in the southern part of Perú under the bilateral agreement with Chile, the government and private sector are interested in creating FFFA or FFLPA in central and northern Perú to increase exports. Fruit production in Perú is developed in more than 176,000 ha in three ecological areas: the coastal area, the central interandean valleys and the eastern jungle. In the coastal areas, there is a significant potential to create FFFA, due to the geographical isolation of the fruit-producing valleys. Mango, grapes, mandarin and other citrus are the main fruits targeted for export. The total valule of fruit production in Perú is more than US$ 500 million per year. It is estimated that fruit flies are causing losses amounting to US$ 25 million per year, representing approximately 20% of the total losses due to agricultural pests.

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Peruvian Plant Protection Officials are seeking technical assistance from IAEA to re-establish their National Fruit Fly Programme.

Uruguay: In 1989, in conjunction with Argentina and with the support of FAO, Uruguay developed a bilateral TC project to establish an area-wide programme to control the medfly and the South American fruit fly in the important and relatively isolated citrus area of the Litoral along both sides of the Rio Uruguay. Since that time, due to the lack of continuity, organization and technical assistance, the proposed area-wide project was frozen by the two countries. In this area, on both sides of the border, there are more than 50,000 ha of commercial citrus plantations. On average, 18 to 22 applications of insecticide are sprayed per year by the citrus farmers to obtain control of medfly. This control programme is costing US$ 5 to 6 million annually. Furthermore, as a result of the excessive pesticide use, there are unpredictable and unquantified side-effects on human health and the environment.

This project along the Rio Uruguay area is technically, economically and environmentally feasible by applying an area-wide control strategy to control the pest over the entire citrus area, including the highly infested fruit trees in urban areas. An important reduction in the use of insecticides and, in consequence, the reduction of environmental pollution and programme costs are the most important factors to consider in this project.

Presently, the citrus growers organization are requesting from their respective governments (Argentina and Uruguay) support for the establishment of an area-wide control programme using the Sterile Insect Technique to effectively control this destructive pest. The government of Argentina is seriously thinking of moving resources to this area and of providing support from the medfly eradication programme in Mendoza.

Bolivia: The Government and private sector of this country are interested in developing fruit production as an alternative to coca production. In this case, tropical and subtropical fruit production for export, such as chirimoya, pineapple and citrus, is the general objective. However, fruit flies, mainly medfly and SAFF, are presently the most important quarantine pests that have to be controlled to establish FFLPA and, in some limited cases, FFFA.

Brazil, an important fruit-producing country in South America, is seriously looking for environmental-friendly alternatives to control fruit flies in the large citrus areas of the country, because pesticide residue on the fruit is becoming an important problem. Plant protection officials and scientists are strongly thinking of developing biological measures, such as the production and release of large quantities of parasitoids over the fruit fly infested areas. This biological method includes the production of parasitoids on mass reared and irradiated fruit fly larvae. The combination of this technology with SIT will be an excellent alternative to reduce pesticide application and effectivelly control the pests. In Brasil, there is already a fruit fly free area, allowing the exportation of melons to the northern hemisphere. Furthermore, in the southern part of the country, important agricultural development programmes are in progress, including the expansion of apple and citrus fresh fruit production. In this area, the South American fruit fly and medfly are the targeted pests to be controlled.

In Colombia the mango growers are implementing a programme to export this subtropical fruit. Due to the presence of fruit flies, mainly medfly, mango and other tropical fruits can not be exported to the important NAFTA market. For this reason, the fruit growers

are making serious efforts to overcome the quarantine restrictions imposed by the USDA. For the moment, they have built the infrastructure to apply the post-harvest quarantine treatment for mango (hot-water), but fruit fly suppression actions have to be taken at the pre-harvest level. This means the creation and certification of FFLPA. With an internationally recognized FFLPA, the mango growers would then be able to proceed with the fruit to the postharvest treatment so that it finally can be accepted by international markets. For this reason, Colombia is very interested in receiving technical support to establish an effective fruit fly control programme in those areas selected for fruit and vegetable exports. The regional project would supply this required technical assistance.

There are other countries in the region that are independently developing concrete actions against fruit flies. In the future, Ecuador and Venezuela would be very interested in joining a subregional fruit fly project.

In view of the above, a regional fruit fly strategy could be envisioned in which the present succesful national TC projects in Chile and Argentina could be the local sources of technology to be transferred to the neighboring countries.

DAMAGE EVALUATION OF *Anastrepha fraterculus* (WIEDEMANN) (DIPTERA: TEPHRITIDAE) ON FIVE APPLE CULTIVARS UNDER LABORATORY CONDITIONS

(Abstract)

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The apple production losses in southern Brazil caused by the attack of the fruit fly *Anastrepha fraterculus* can reach up to 100% in some years. Its control demands intensive systematic sprays of insecticides, which increase production costs and affect environmental quality. In terms of integrated pest management, the use of resistant cultivars represents one of the most important alternatives to control this apple pest. With the objective of identifying sources of host plant resistance, apple fruits of different cultivars from the Clonal Germplasm Repository of the EPAGRI Research Station of Cacador were tested. The experiment consisted of 5 treatments (cultivars) with 5 replicates. Fruits at the harvest stage were used. The fruits were placed in boxes (40 × 110 cm), where they were exposed to oviposition by the fruit fly. After infestation, fruits were left on shelves at room temperature for 10 days in order to evaluate the damage level according to the following scale: 1 = fruit without attack; 2 = fruit with punctures and/or deformation without galleries; 3 = fruit with punctures and/or deformation and galleries; 4 = fruit with punctures and/or deformation, galleries and larvae. The Gala cultivar was the most susceptible, with an average damage level of 3.4, differing from the cultivars Fuji and Royal Red Delicious (damage levels of 1.6 and 1.2, respectively). The Belgolden and Sansa clones presented intermediate damage levels. *A. fraterculus* preferred to oviposit in the Golden Delicious group compared to the Delicious group. These studies suggest good possibilities for reduction of insecticide sprays to control the fruit fly in the cv. Fuji, as well as the incorporation of resistance factor in apple cultivars.

BEHAVIOUR OF *Anastrepha fraterculus*

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Abstract

BEHAVIOUR OF *Anastrepha fraterculus*.
A number of experiments and observations on the behaviour, host associations, attractants for adults and pupation of the South American fruit fly *Anastrepha fraterculus* (Wiedemann), conducted under field or semi-natural conditions are presented here.

1. INTRODUCTION

This paper presents a summary of experiments on *Anastrepha fraterculus* (henceforth AF) conducted under field or seminatural conditions.

In southern Brasil between parallels 23 and 32, a region with a subtropical climate, AF is the totally dominant species, comprising as high as 95-97% of all *Anastrepha* trapped/captured, and it is the only fruit fly species of economic importance, that is, a pest.

In this region there are many and various fruit crop species available (in space and time) throughout the year. The Mediterranean fruit fly, *Ceratitis capitata*, is present in this region, but only as an "urban" resident. In the cities, both species (AF and *C. capitata*) coexist and infest common fruit species. Fruits of guava and peach, for example, are infested by both species in a single fruit. However, *C. capitata* does not live in the rural areas. Peach and orange orchards in suburban areas are infested by *C. capitata*, but when they are located around 10-15 km from the urban areas, only AF is found infesting these fruits.

In São Paulo state, both species coexist in many fruit species orchards (e.g., orange, coffee). Intra- and interspecific competition occur at the larval stage, and factors such as temperature may be determinants for their relative frequency in nature. It was suggested that temperature is a factor that would confer an adaptative advantage to one or the other species and determine a better rate of survival. Temperatures in the vicinity of 16°C would confer an advantage for *Ceratitis* over AF. The threshold temperature favoring AF would be around 22°C, above which, AF develops better than *Ceratitis*.

In Argentina (Bella Vista, Corrientes), *C. capitata* and AF coexist in citrus orchards (small populations of both species), but cultivars of early and middle maturation are more infested by AF and later cultivars are infested by *C. capitata*. *C. capitata* is considered the most damaging species.

In southern Brasil, *C. capitata* does not attack citrus in rural areas, but only in urban backyards. AF only sporadically infests citrus in the rural areas, despite a large number of varieties and acreage of these fruits.

Behavior of AF as a potential pest species is still far from understood.

2. ADULT BEHAVIOR

Studies on adult behavior and activity were conducted in Pelotas, Brasil, lat.31 S, long. 52 W, altitude from 7 to 100m). Adults of AF emerged during all months in the fall and winter, indicating that this species has no winter diapause or quiescence in this region. In the fall (April and May), the pupal period (until emergence of adult) takes from 31 to 47 days and, in winter (June, July and August), it takes 31 to 167 days.

From pupae formed under guava trees, adult emergence takes 27 to 41 days, but the highest number emerged at 34 days after pupation (April 30). Males emerged first, but at the end of the day, an equal number of males and females had emerged. Adults (females and males) show flight activity throughout the year. During the winter months (June, July, August), normally very few are captured, and increased capture is associated with a day or days of abnormally higher temperatures. This tells us that adults flies are alive and in the environment during the winter time, although we do not know where they are located, if they are isolated or agregated, if there is migration, etc.

In September, the adult population starts to increase, peaking in November through December and drastically decreasing in January and February (summer months). Highest captures of AF occurred during November and December.

In peach orchards, two peaks are typical. The first is during the last week of October and the first-second week of November, when growers generally spray. The second peak occurs after harvesting, in the third-fourth week of December and the first-second of January. This temporal variation depends on the cultivar composition of the orchard.

There is no relation between time of maturation or harvesting and adult capture (harvesting of peach occurs in December and January). In peach, AF only infest fruits during the "swelling" period or stage, i.e. 25-20 days prior to the harvest maturation point. In peach, AF has only one generation per cultivar. Considering alternative hosts, AF (in Pelotas) has full conditions to develop 5 to 7 generations per year. Highest capture of adults occurred in the period between 11:30 to 19:00 hours. Lowest captures occurred during morning and nocturnal periods. No capture occurred on rainy or windy days or, at least, in days with afternoons under these circumstances. In general, AF uses the morning hours for reproduction, afternoon hours for locomotion and night hours for resting. AF was captured from 1,5 to 10 m above the ground. A vertical differentiated capture occurred at 4 and 6m, with highest values at these heights.

Fruits were equally infested at 2, 4, 6, 8 and 10m above ground level and no difference occurred among these heights. This suggests that AF adults display full activity - flying and infesting fruits - from 2 to 10m above ground level.

No study of horizontal flight distance or dispersion capacity (± migration patterns) of AF was conducted. Colonization and dispersion of adults of AF in peach and apple orchards had different patterns. In peach, fruit presence (especially close to ripening) had a positive correlation with occurrence and distribution. In apple, no effect of fruit or/and its condition was observed. In peach orchards, adults seem to be "residents" in the area but, in the apple orchard, they are "in transit," probably living in surrounding vegetation (especially in forests). Presence of native forest had a detrimental effect on the colonization and distribution of adults

in apple orchards, but not in peach orchards. In apple, adults began to occupy the orchard in areas adjacent to forest and concentrated their presence in forests and surrounding areas. In peach, flies were massively captured within the orchard, but not in the forest or nearby.

Apple is a new host for AF (approximately 20 years). It is possible to speculate that this fruit fly species is in a coevolutionary process and is adapting to explore and use apple as a host. Observations of the diel pattern and location of activities of AF on host trees, with and without fruit, and non host trees without fruit, revealed: at dawn (6:00h) both sexes were at rest on the bottom surface of leaves near the top of nonhost or host trees. Sexual activity (male calling) began at 7:00h and ceased before 11:00 h. It occurred on nonhost as well as hosts, with all observed copulation initiation and mating occurring only near the top of a tall nonhost tree (Malavasi et al 1983). In guava and peach trees, copulation movements and mating were observed (Salles, L.A.B., personal observation). Flies were most abundant on major host trees that had fruits. Feeding and oviposition began at 8:00 h, peaked from midmorning to midafternoon and ended before 18:00 h. Flies observed at dusk (18:00 h) and afterward were either at rest on the bottom surface of leaves near the tops of trees or were in flight toward tree tops. Adults seem to have a pattern of movements on the tree according to time of day, either going up and downward.

Diurnal courtship, activity and mating system of AF was studied in caged trees, in Pelotas, Brasil (Lima, I. & Salles, L.A.B.). A brief summary is presented: Flies are feeding and grooming throughout the light period and ceased with the onset of darkness. Locomotory activity was also almost exclusively diurnal. There was a peak in locomotion (mainly walking) at the beginning of the dusk period. Most of the flies remained still or moved very little in the dark.

Sexual maturation of male flies began at the age of 5 days. Males younger than 5 days showed no courtship behavior. At dawn, mature males repeatedly rotated their bodies through 360°, while rapidly fanning their wings. However, they did not try to copulate and did not approach other males or females. This is part of a calling display which ceases after one hour of full light conditions. The duration of male calling activity was increased to nearly four hours when they reach 8-9 days old. Males were seen calling either in isolation or in small groups of 3-4, usually at the top of the cage. During the dawn period, calling activity was observed in 60% of the males in the cage. It increased to nearly 100% at the beginning of the period of high light intensity, when most mating initiation occurred and calling males had selected a position in the cage, either isolated or in leks. Calling remained at a high level during the next two hours.

Most of the mating observed occurred during the dawn period and continued through two hours of full light conditions. All recorded mating took place within the first two hours of light. Copulation duration time was 60-80 minutes.

Female flies reached sexual maturity at 11 days old. Females younger than this were not seen copulating. Feeding occurrence was mainly during the period between 4 and 8 hours of light conditions. Mated females were not observed ovipositing during the dawn period but immediately after the beginning of full light conditions. However, oviposition occurred throughout the day and was observed even during the dusk period. The relationship between fruit phenology and infestation by AF was studied only for two fruit species, peach and apple. In peach (5 cultivars), infestation occurs only at and during the fruit ripening (swelling) stage. This stage, for the studied cultivars, occurs close to 25-20 days before the first harvest time.

It is concluded that AF infests peach only during a short period of time. Obviously, this fact must be strictly considered in any pest management program, and it makes it much easier to establish control measures, especially timing insecticide use for larval control. In apple (Gala cultivar) infestation with live, developed larva (II, III instars) is extremely rare. We assumed that very high mortality occurs during the first larval stage.

Females are usually strong and intensively oviposit (=puncture) into apple fruits. Fruits 1-2 cm in diameter are already punctured and damaged by females. This damage is irreversible, producing a skin deformation with a consequent market value depreciation. When small fruits are punctured they may drop, reducing future production. Larval development of AF is observed extremely rarely in apple. It occurs only in overipe fruits, usually fruit that have dropped to the ground. (Salles, L.A.B., personal observation: in ten years of surveys, I have never found a fruit infested with mature (III instar) larvae in commercial orchards).

External damage on fruits is very severe due to numerous punctures per fruit. Fruits had an external appearance resembling the figure of a "moon surface." Internal damage occurs just under and around a puncture site. This damage became like a corked tissue, similar to the damage or symptom of the bitter pit disease. This situation, due to actual behavior of females of AF on apples, makes it difficult to take actions for its control. A long time period of control actions (> 90 days) is required.

3. HOSTS

In southern Brasil, 83 fruiting tree species were evaluated to establish their status as hosts for AF. Among these, 59 species were not infested by AF and 24 were infested. Of the infested species, 9 were classified as heavily infested and are considered to be alternative hosts. Despite this classification, AF has a considerable number of hosts, and they are distributed throughout the year: Plum, blackberry, araca (*Psidium* sp), wild cherry, guava, feijoa, guabiroba (*Campomanesia xanthocarpa*), jaboticaba (*Myrcia jaboticaba*), sour orange, common orange, laranja de umbigo (*Citrus* sp), kinkan, lima (*Citrus linetoides*), lemon, apple, maria preta (*Diateropteryx sorbifolia*), mata olho (*Chrysophyllum gonocarpum*), strawberry, loquat, pear, peach, wild peach, and pitanga (*Eugenia uniflora*).

It is interesting that several mentioned "traditional" hosts for AF were not infested in southern Brasil, including avocado, coffee, kaki, fig, papaya, mango, passion fruit, quince, pecan, grapefruit, grape and uvaia. As already studied and proposed, AF may have differentiated host patterns, probably due to the existence of biological races or biotypes, adapted to local conditions. Larval and pupal development of AF was evaluated on different hosts. The average number of pupae per fruit varied from 0.7 to 9.9. Hosts that produced more pupae were feijoa, loquat, peach, and plum. Bigger and heavier pupae were obtained from wild cherry, brazilian cherry, guava, plum, and peach. In general, those hosts that produced a larger number of pupae were not those that produced bigger and heavier ones. Another study stated that the average number of larvae per fruit was between 1-5 in large fruits (guava, peach and loquat) and it was <1 in small fruits (Surinam cherry).

4. ATTRACTANTS FOR ADULTS.

Some studies were developed to determine the efficiency of different attractants for adult AF. Most studies were concentrated on food attractants. Most studies of attractants for

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adults had monitoring as the main goal. No visual responses occurred to colors, forms and their combination. Yellow rectangles were more attractive for capture of AF. Spheres seem to be much more attractive to females than males. Traps using color and format are not used for monitoring AF.

No acoustical attractant trap was tested for AF and no information on this aspect is available. Lures based on pheromone (or any semiochemical) are not available to capture adults of AF. Pheromone studies of AF have been initiated in Southampton University (Dr. Philip Howse, Ivanildo Lima), but the current stage of their development and advances have not been reported. Adult attractants (food) already used were vinegars, protein in hydrolyzed proteins, molasses and several fruit juices. Fruit juices seems to be the best attractants.

The most common juices (probably efficient) are peach, grape, orange, feijoa, and guava.

The ideal attractant for adult AF is still to be discovered and/or to be indicated. Probably there is no one best or ideal, but local conditions (e.g., cost, ease of preparation) could determine their use and advantage. Hydrolyzed protein was recommended as a "universal" food attractant for fruit flies, including AF, but in southern Brasil, for example, is much easier and cheaper to use peach or orange juices instead. Traps used for AF capture are the traditional McPhail glass trap, modified McPhail plastic trap and several homemade traps, especially those made with disposable plastic containers. Again, cost and ease of preparation suggested which trap should be selected, of course, among those evaluated.

5. PUPATION

Pupation behavior of AF was studied in the laboratory and field. Larvae complete their development in a single host fruit and leave it a few hours (1-2 hours) before pupation. Larvae do not come to the soil by any part of the tree but by "jumping" out of the fruit and falling on the soil surface, however high the fruit is. We have measured that larva have "jumped" onto the soil and entered it in few minutes (2-5 minutes) from fruits as high as 11 meters high on the tree (loquat tree).

The time spent by larvae to enter the soil for pupation also depends on the soil characteristics, such as humidity and compactation level. Larvae spend time crawling and "looking for" a suitable place to get into the soil. When the fruit are on the soil, the larva leaves and crawls away from the fruit at least about 5 cm to pupate. This is a critical time for larvl predation. In areas with fire ants, heavy predation occurrs. Under a loquat tree canopy, for example, the number of fire ant nests increases when fruits begin to fall on the ground and larvae are emerging.

Under the guava tree canopy (natural compaction conditions), all puparium were found in the first 6 cm of the soil, but most of them were located in the first 4 cm. When soil was cultivated at 10cm of depth, puparium were found this deep, but between rows, with compacted soil, they were localized only up to 4 cm deep. In the laboratory, puparium formation was related to the depth of the soil layer and its level of compaction. In soft soil (no compaction) puparium were found at 18 cm, but most were concentrated in the first 10 cm. When soil was artificially compacted, most puparia were found in the first 4 cm. There was a direct response of the depth of pupation to the level of soil compaction. Level of soil compaction did not influence time to emergence and number of adults emerged.

BIOLOGY OF *Anastrepha fraterculus*

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Abstract

BIOLOGY OF *Anastrepha fraterculus*.

This paper presents available (published) information on specific abiotic factors, such as temperature, humidity, and lighting on the life cycle of *Anastrepha fraterculus*, henceforth AF. AF displays holometabolous development: egg, larva (development is completed in three instars), pupa and free living adults.

1. TEMPERATURE

Temperature has a detrimental effect on all of these phases. Thermal requirements for development of AF are shown below.

Basal temperature (°C): minimum temperature for its development

Egg	9,2	6,3
Larva	10,3	
Pupa	10,8	
Adult	---	
Life cycle	10,7	

The minimum developmental temperature (= lower lethal) for all stages of AF is very close to 10°C. Eggs are able to develop at lower temperatures, perhaps as low as 6°C.

Threshold development (°C): range of temperature to complete phase/life cycle development of AF

	Lower	Upper
Life cycle	15,3	26,8
Egg	11,9	13,2
Larva	13,8	18,8
Pupa	14,7	21,4
Adult	14,1	35,7

To complete development, temperatures must range between 15 to 27°C. This is the range of effective temperatures that promote development of AF.

Cumulative heat development (thermal constant) (= degree days): amount of hours above 10,7 °C, necessary for development of AF phase/life cycle

Egg	52,2	61,2
Larva	161,4	
Pupa	227,7	
Life cycle	430,6	

Life cycle (days) of AF at different temperatures

At: 25 +- 0,5 °C; 60 +- 10%RH; 14 h photophase

Pre-oviposition		15-35*	(22,7)**
Oviposition	0-150	(79,1)	
Egg/female/day		0-40	(25,2)
Egg/female/total	0-979	(394,2)	
Incubation	1-3	(2,7)	

* range ** average

At: different temperatures; 70-80%RH; 14 h photophase

	10° C	15° C	17,5° C	20° C	22,5° C	25° C	27,5° C	30° C	35
Egg	no	10,3	7,9	4,7 4,8 5,3	4,2	3,0 2,8 3,2	2,6	2,3 2,6 2,9	no
Larva	no	34,5	22,5	16,2 17,5 19,0	14,8	11.0 12,7 14,0	11,3	14,0 18,7	no
Pupa	no	43,2	37,1	26,0 27,3 32,1	18,8	10,0 14,1 15,3	13,0	11,8 13,5	no
Adult	no	128,7		104,1		55,5			no
Life Cycle	no	88,0	67,5	46,9 49,6 56,4	38,8	23,8 29,8 32,5	26,9	28,1	no

Development (days) of immature stages at different temperatures

	Egg to Pupa	Pupa to Adult	
		Female	Male
5 °C	no	no	
10	no	no	
15	38-56 (45,2)	no	
20	26-38 (31,7)	59-72 (63,0)	59-76 (65,6)
25	11-21 (15,6)	26-31 (30,0)	28-35 (31,3)
30	32-45 (38,4)	no	
35	no	no	
40	no	no	
45 °C	no	no	

Pupal development (days) at different temperatures

	Days to emergence	% emergence
17,5 °C	35-42 (38,6)	86,5
20	23-32 (27,3) 59-72 (63,0) 59-76 (65,6)	89,7
22,5	17-21 (19,3)	100
25	11-21 (15,3) 26-31 (30,0) 28-35 (31,3)	100
27,5	12-16 (13,8)	97,5
30 °C	10-21 (13,5)	98,2

Larval development (days) at different temperatures

	First instar	Second instar	Third instar	Total
17 °C	6-8	4	11	21-25 (22,5)
20	3-4	3-4	8-12	13-23 (17,5)
22,5	2-4	3	6-12	12-20 (14,7)
25	2-4	3	6-8	12-14 (12,7)
27,5	2-3	1-3	5-10	10-15 (11,2)
30 °C	2-6	1-3	6-9	10-16 (14,0)

Oviposition at different temperatures

	Oviposition (days)			Number of eggs
	First	Last	Period	per female
5, 10, 15 °C		no		---
20	19	69	50	67-274 (171)
25	7	53	46	341-446 (408)
30	7	69	62	159-276 (236)
35, 40, 45 °C		no		---

1.1. Specific bibliography of AF and temperature

Baker, E.W. 1945. Studies on the Mexican Fruitfly known as AF. J. Econ. Entomol. 38(1): 95-100

Machado, A.E., Salles, L.A.B. & Loeck, A.E. 1995. Exigencias termicas de AF e estimativa do numero de gerações anuais em Pelotas, RS. An. Soc. Entomol. Brasil 24(3): 573-578.

Martins, J.C. 1986. Aspectos biologicos de AF em dieta artificial sob diferentes condições de temperatura e fotoperiodo. Dissertacao de mestrado. ESALQ. Piracicaba, SP. 80p.

Salles, L.A.B. 1993. Efeito da temperatura constante na oviposicao e no ciclo de vida de AF. An. Soc. Entomol. Brasil 22(1): 57-62.

2. ATMOSPHERIC HUMIDITY (= RELATIVE HUMIDITY)

No study was conducted to study the influence of free air humidity (moisture) on any life stage or phase of AF. Only adults are supposed to be directly influenced by air humidity (moisture). Eggs and larvae are dependent on host humidity or moisture content and pupae are protected by the puparial case with an indirect effect of the soil. Puparia formed on the soil surface or just beneath it may experience a direct influence of air humidity.

Most studies on the biology of AF were developed under the range of 60-80% relative humidity. However, one study was conducted to measure the effect of soil humidity (moisture) on pupal and adult emergence of AF (Salles, L.A.B. et al. 1995. An. Soc. Entomol. Brasil 24(1): 147-152).

Pupal period and emergence of AF at different temperatures and soil moisture

Moisture (%)	Temperature (°C)			
	15	20	25	30
2,6	55-59*	38-40	15-18	21
	31,3**	33,0	79,1	2,3
9,0	54-56	34-38	14-16	21
	86,2	91,1	96,2	3,6
12,8	55-60	34-35	14-16	21
	96,2	91,1	89,8	1,1
17,9	55-59	34-35	14-16	21
	88,2	92,4	94,9	4,7
24,3	56-59	35-38	14-17	21
	75,5	78,4	92,4	5,3
28,5	56-59	35-37	15-18	21
	61,8	70,9	84,7	3,3

* first and last day that emergence occurred
** percentage of emergence

An effect of temperature on the pupal stage was noticed. Again, faster development occurred at 25°C at any soil moisture level. At 30°C, at all soil moisture levels, emergence of all adults occurred on the same day (21 days). Adult emergence occurred at very low (2,6%) and at high (28,5%) soil moisture levels.

At 15, 20 and 25 °C, no differences occurred in the percentage of emergence at any level of soil moisture. Pupae did not survive at 10 and 35°C. At 30°C, emergence was very low (1,1-5,3). Soil humidity or moisture had no direct effect on the pupa, only temperature.

3. LIGHT PHOTOPERIOD

Number of days for larval and pupal development of AF at different photophases (=hours with light)

Photophase	Larva	Pupa
0	12-30(20)	14-30(18)
6	12-28(21)	14-18(14)
12	12-30(22)	14-16(15)
14	12-30(21)	14-18(15)
18	13-30(24)	14-30(17)
24	14-30(27)	14-33(19)

Number of days for larval, pupal and life cycle of AF at different photophases

Photo phase	Larva	Pupa	Life cycle	
			Female	Male
0	11-22(16)	15-24(19)	26-35(30)	26-35(30)
6	13-22(17)	21-27(24)	34-40(37)	32-42(37)
10	13-22(17)	21-29(25)	34-42(38)	34-42(38)
14	11-22(16)	20-24(22)	31-35(33)	27-38(32)
18	15-24(19)	16-24(20)	31-39(35)	31-38(34)
24	13-20(16)	14-20(17)	27-33(30)	27-34(30)

Adult longevity (days) of AF at different photophases

Photophase	Female	Male
0	7-100*(42)	10-100*(54)
6	67-100(87)	5-100(37)
10	5-100(44)	5-100(53)
14	5-100(51)	5-100(44)
18	10-100(79)	5-100(67)
24	7-100(60)	65-100(84)

* adults were observed up to 100 days only.

Oviposition of AF at different photophases

Photophase	Period of oviposition	Average eggs per female
0	59-71(65)	348
6	51-75(67)	437
10	36-73(76)	375
14	43-64(50)	345
18	44-60(52)	278
24	24-75(47)	369

Emergence of AF at different photophases

| Photophase | Period (days) | | | % emerged |
	First	Last	Duration	
0	15	15	0	90
6	17	20	3	94
10	17	20	3	100
14	15	17	2	94
18	15	17	2	95
24	13	15	2	76

There is no direct influence of the day length or photoperiod on duration of any life cycle phase of AF or on its performance. In nature, adults are the only stage directly influenced (if so) by light, and no effect of any measured life event occurred through all six photophases studied. This situation suggests a strong independence of this species to photoperiod duration. If this is true in nature, this species should have the same development under short or long day conditions.

3.1. Specific bibliography on photoperiod

Martins, J.C. 1986. Aspectos biologicos de AF ... em dieta artificial sob diferentes condições de tamperatura e fotoperiodo. Dis. Mestrado. ESALQ/USP. Piracicaba. 80p.

Salles, L.A.B. 1993. Influencia do fotoperiodo no desenvolvimento de AF. An. Soc. Entomol. Brasil 22(1): 47-55

4. LIFE EXPECTANCY OF ADULT AF

At 25°C, 70-80%RH, 16 h of photophase (2000 lux), the maximum longevity period for females and males was the same= 161 days. Fifty percent mortality for the female and male population (of fifty couples) was reached at 15 and 18 weeks, respectively. Adult AF, at least in the laboratory, have a long life span. Lima el al (1994), working with another AF population and conditions (26 +- 2°C, 60 +- 10% RH, 13 h photophase of 1600 lux) found a mean longevity for males of 196 days and for females of 172 days. Flies which lived longest

did not achieve the highest number of matings. Males that mated twice during their lifetimes lived for 276 days while a female that mated only once lived for 269 days. Flies ceased mating at a mean age of 100 days. It is not clear if the number of matings influence life psan for either females or males.

5. OVIPOSITION

Because oviposition is one of the most, if not the most, important biological eventsof a fruit fly's (including AF) life, additional details will be considered:

The pre-oviposition period has been determined by many researchers. It varies from 7 to 30 days (7, 9, 15, 22, 30 days). Certainly, these drastic differences are determined by unknown factors, probably the origin of AF population or biotype, adult food, experimental conditions and procedures. In nature, this period has not yet been determined. AF lays eggs without mating, at least, when confined in cages or in experimental containers. Obviously, these eggs are infertile and no larvae are produced.

Oviposition period varies from 65 to 80 days

Martins (1986) determined that a decrease in egg viability was positively correlated with oviposition period. At 10 days into the oviposition period, females are laying close to 20 eggs per day with 70% viability; at 40 days into oviposition, they are laying close to 10 eggs with 20% viability and at 80 days into the oviposition period, oviposition averaged only 5 eggs per female per day with less than 5% viability. This is an extremely important factor to be considered in a mass-rearing process. Young females lays more viable eggs. Thus it would seem to be unproductive to keep females older than 50 days in oviposition cages, for example. Egg production varies from 278 to 437 (313, 345, 369, 375, 384, 394) eggs per female.

Oviposition behavior of AF is different in laboratory and natural conditions (Barros et al 1983). Under natural conditions, four stages were characterized while, in the laboratory, this pattern was disturbed, including, in most cases, the absence of any sequential stages. Normally, AF lays one egg per puncture (= ovipositor introduction). However, it may puncture without laying an egg. On artificial fruits or oviposition devices, two eggs are sometimes found clustered, probably laid together. This behavior is very important and should be considered, because it gives to the "adult female" the status of a pest. In certain fruits (e.g., apple, pear) economic damage is caused by the puncture itself. When these fruits are punctured, irreversible damage is produced on the fruit skin and in the first layers of the pulp. At least in rearing cages, AF lays eggs without a proper substratum (e.g., fruit, artificial substratum). Eggs are laid on the surface of different cage parts. We don't know if this behavior is maintained under some adverse natural conditions (e.g., if there are no suitable fruits for oviposition). Under some conditions, females have so many eggs in their abdomen that it is enlarged to almost twice its normal size, has become translucent and the fly seems to be about to blow up. Probably, under these conditions, females may become promiscuous in their oviposition behavior.

In Pelotas (south lat. of 31) females are gravid throughout the year and lay eggs whenever fruits are available. In winter, very few fruits are available, but most are infested. Probably, temperature is not a limiting factor for egg production but for oviposition. If this is true, gravid females and/or females with eggs, will copulate and lay eggs any time that the temperature reaches some threshold, perhaps between 12 and 15°C.

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NOTES ON THE PRESENT SITUATION OF
Anastrepha fraterculus IN ARGENTINA

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Abstract

NOTES ON THE PRESENT SITUATION OF *Anastrepha fraterculus* IN ARGENTINA.

Behavioural and karyotypical results from *Anastrepha fraterculus* samples received in our laboratory during the period 92-94 are discussed. The work represents the partial situation of this pest in Argentina. Data from guava fruits indicate 1) that the oviposition behaviour does not agree with the autocontrol mechanism via ODP previously described, 2) that a selection mechanism associated to fruit size and quality exists, allowing the female to choose the oviposition site, 3) if different varieties of fruits are available, then the fly shows preference for one of them.

The oviposition behaviour in samples of peaches is different from that observed on guava samples and agrees with the ODP mechanism. In some localities, *A. fraterculus* and *C. capitata* are sharing the same substrate or fruit (same unit). Samples from commercial oranges do not show evidence of *Anastrepha* attack.

A more frequent karyotype *fArg 1* (wild type karyotype) as well as high karyotypic variation, mainly measured through the polymorphism of sexual chromosomes, are present within different geographic populations sharing the same host species. To date, we do not have enough information to associate a particular behaviour to a karyotype. The chromosomal analysis is merely descriptive.

1. INTRODUCTION

Anastrepha (Schiner) is the neotropical tephritid genus which most attacks fruits of natural and cultivated species in America. Steyskal [1] recognizes 155 species, but only six of them are economically important [2].

The South American fruit fly *Anastrepha fraterculus* (Wied) is considered to be native of the Galapagos Islands [3]. It has a wide geographic distribution, from the Rio Grande Valley, Texas, U.S. to Chile and Argentina. According to Stone [4], it inhabits between the 27 N and 35 S parallels. Its larvae live inside fruits of different species and it is a very important pest in Argentina.

Maddison & Bartlett [5] indicate the presence of the genus *Anastrepha* in this country even in the region of Patagonia. Except for accidental sporadic introductions, this information is not too likely because the eco-climatic conditions below the 38S parallel become a barrier for *Anastrepha* development.

Patagonia is a desert region composed, in the non mountainous zone, by two habitats described as follows: in the North, arid-semi arid temperate climate (annual maximum 18°C) and in the South, plateau cold arid (annual mean 6-12°C). The natural vegetation corresponds with a pasturing degraded rockrose.

Cultivated areas are found at oases, on the hillsides of thawing rivers and are separated by more than 100 Km from each other [6]. Aluja [7] states that the genus does not occur in the South region of Argentina. Blanchard [8] describes the presence of 35 species and 2 varieties of the genus *Anastrepha* in Argentina. These results can be summarized in the

following way: the description of 60% of the species was based on samples of 1 to 7 individuals. Further work [1] synonymizes 2 of them and assembles another seven species into the complex *A. punctata*. Among the remaining species, 11% are designated as using *Passiflora* sp. as hosts, 6% as using vegetables as hosts, 12% without recorded hosts and only 11% as using fruits as hosts: *Anastrepha fraterculus, A. punctata, A. schultzi* and *A. pickeli*. Aluja [7] describes only seven species of the genus as pests, three of which would be present in Argentina: *A.grandis*, specific for cucurbitaceous hosts, *A. serpentina* for sapotaceous hosts and *A. fraterculus*, which attacks a wide diversity of fruit species. The existence of this latter species in this country was confirmed by Dr. Weyenbergh in 1874 under the synonym *Anthomya persicorum*, a fact recorded by an anonymous author [9]. This anonymous paper from the Argentinian Ministry of Agriculture indicates that, up to that date, *Anastrepha fraterculus* was the only species of the genus present in the country, "existing more abundantly in the Provinces of Tucuman, Salta and Jujuy where it produces great damage during those years favorable for its evolution." Generally, the fruit fly (common name used here) prevails in years of little climatic variation, and jeopardized damage, diminishes noticeably during the years with sudden changes of temperature. That publication points out "that it prefers thin peel fruits such as peaches, cherimoya, damsons, guava fruits, figs and caqui, but that it also attacks citrus: orange, mandarin orange, kumquate and grapefruit, when a diminution of the first type of fruits or a superabundance of autumn flies, oblige it to attend on thick peel fruits to perform oviposition;" this is the reason why it is sometimes called the orange fly or orange worm. That report determines "that the attack begins in damsons during spring, it continues in peaches, and when this species finishes fructification, then there is no fruit which does not receive perforations from this fly, despite its larvae cannot develop in all the varieties. The cherimoya and guava fruits are successively utilized to feed two generations of flies. Next, they apply for caqui until the time oranges and mandarin oranges are about ripening. Most of the eggs oviposited on orange peel result destroyed by the action of essential oils, while many little larvae die due to the lack of food before boring the thick peel and reaching the pulp." The report continues: "Generally the development of *Anastrepha fraterculus* worm inside citrus fruits is slower than that taking place in other plants; this is due on one hand to the qualities of the fruit itself and on the other hand to the relatively low temperature prevailing during the period of maturation of these fruits. The activities of worms completely paralyze on winter coldly days but on warmy days, larvae renew their development. Climatic conditions as drought and high temperatures stop the development of the flies. It was demonstrated that many larvae and pupae soon die when temperatures are higher than 37°C. The same is also true for new born adults. Instead, this insect can perfectly resist low temperatures of 7°C below cero, and the strong hoarfrosts do not have any effect on them. These flies are 1) indirectly favoured by rains, because consequently a greater development of trees takes place and 2) directly because earth becomes softer favouring adults to get rid of their underground bundle." Stone (1942) (taken from Annals of Argentine Agriculture, 1874, 2:165), explains that this species had already been reported from Argentina by Dr. Weyenbergh as *Anthomya frutalis*.

Anastrepha fraterculus populations from different latitudes and ecological niches have been studied systematically, cytologically, biochemically and ethologically.

The review by Baker et al. [10] distinguishes Mexican *A. fraterculus* from that of the South region because it does not attack citrus and through morphological differences. These authors observed differences in the main host and differences in wing pattern. Nevertheless a complete investigation of host records in both areas showed that not only South American but also Mexican populations attack citrus as well as other hosts [2].

Karyotypic variation within Brazilian and Mexican populations is demonstrated when comparing the works of Mendes [11] & Bush [12]. Bush suggests that it is not possible to identify differences among *A. fraterculus, A. mombinpraeotans* (considered a variety of *A. fraterculus* by Blanchard) and *A. distincta* [12]. Four distinguishable karyotypes have been described for different populations of Brazil, taking into account sexual chromosome diversity [13]. This work suggests that these are sibling species. Afterwards, karyotype 3 was assigned to *Anastrepha sororcula* [14].

Results for the Argentine Republic are discussed by Lifschitz et al. [15] and Manso & Basso [16]. The most frequent wild type karyotype *fraterculus Arg. 1* [17] present in all Argentine populations, is different from those described for Brazilian and Mexican populations [11, 12, 13]. Another cytological study performed on peaches from Montecarlo, Misiones Province, demonstrates that the mentioned karyotype is present at a high rate [16], but a chromosomal configuration (X2) which characterizes, up to now, this population, is also present [16, 17], along with other chromosome polymorphisms

Biochemical studies performed on 11 enzymatic proteins in 13 *Anastrepha* species from 16 populations of *Anastrepha* taken from different host fruits and areas of Brazil suggest the existence of a *fraterculus* complex, heterogeneous and formed by four cryptic species occupying a wide geographic distribution [18]. The *fraterculus* group from Brazil contains the following species (identified, according to Morgante et al. [18], on the basis of female gentalia morphology [19]): *fraterculus, obliqua (mombinpraeoptans), suspensa* and *antunesi*, which, based on other aspects of their morphology, are indistinguishable but biochemically they present differences. Later, *A. sororcula* is included in this group [14].

Another isozyme study of eight populations from different hosts in the same area explained the low variability observed among them based on habits of reproduction and polyphagy of this multivoltine species [20].

Later surveys [21], performed on populations from different South American countries, applying similar techniques, revealed two distinct groups within the species: a group composed by samples from northern Brazil, coastal Venezuela, Costa Rica and Mexico; the other one formed by the samples from southern Brazil, Venezuelan Andes and Perú. The author postulates the existence of a complex of cryptic species of different phylogenetic origins.

A first isozyme study was carried out on three Argentine populations, analyzed in the present work, infesting guava from Ituzaingo (Buenos Aires), Castelar (Buenos Aires) and Yuto (Jujuy, a locality 1800 km. from Buenos Aires [22]. The authors found low genetic differentiation among them, suggesting that all three belong to the same biological species and supporting the occurrence of gene flow among them.

The use of RAPD/PCR techniques on *A.fraterculus* larvae demonstrated differences between populations from Ituzaingo (Bs.As.) and Horco Molle (Tucumán), both taken on guava shrubs [23, 24, 25].

The females of many phytophagous insects use their ovipositors to deposit and spread out a deterring pheromone on the host after oviposition. This would dissuade other females from ovipositing into the same host fruit. In *A. fraterculus*, this marking mechanism is

supposed to diminish competitive conditions among larvae of different stages into the fruit; as a consequence of the specific autocontrol carried out via this pheromone (ODP), the number of larvae found in the fruit would vary from 1 to 5 [26].

Further variation was found when analyzing mitochondrial DNA [27]: four populations of *A. fraterculus* from different regions of America (São Paulo and Bahia, Brazil; Coast Region and Andean Region, Venezuela) could be distinguished by using 3 restriction enzymes.

The present work contains data taken from samples of different geographic origins of the country during the period 92-94 and analyzes: 1) the behaviour, through different samplings, relative to the presence of *Anastrepha fraterculus*, indicating those cases where this species shares the same host with *Ceratitis capitata*, and 2) chromosome variation. This work is not a review of the subject but the basis to make possible the initiation of subsequent working strategies.

2. MATERIALS AND METHODS

Samples were collected and brought to the laboratory to receive the following treatment:

A) Manageable numbers of fruits were isolated into flasks of 3 Kg containing a base of vermiculite to control problems of humidity caused by decay conditions of the fruits. Flasks were covered with plastic foam. At the time pupae could be seen, all samples were carefully examined to avoid damaging the fruit so as larvae of different stages in the fruit pulp could complete their development. Again, pupae were isolated on vermiculite and were identified by the fruit from which emerged and by the date of pupation. The examinations were periodically repeated until the isolation of all individuals.

B) The rest of the material was put on riddling trays, placed on other trays containing a base of vermiculite which was periodically sifted to isolate pupae. Pupae were then treated in a similar way as described in step A. This mass-conducted material was utilized for cytological and isozyme studies and to obtain adults to try different approaches to artificial rearing.

All this material was managed in the same environment, an underground laboratory, which presents little oscillation of natural conditions of temperature and humidity during the months of sampling from September to April, depending on the fruit and its geographical origin.

The following experiments represent samples from different host fruits and localities of Argentina.

Materials came from different localities.

2.1.1. Locality 1 (L1) in Castelar (lat. 34,5°; long.58,5°), Buenos Aires province, is an experimental field of different species and varieties of fruit trees which ripen gradually: 1) guava fruits, 2) sour fruits, 3) plums, 4) damsons, 5) early peaches, 6) apples, 7) late peaches, 8) pears and 9) guava from October to April; it can be considered a zone of temperate climate included in a radius of 1 km.

2.1.2. Locality 2 (L2) in Ituzaingo, Buenos Aires is a family garden with only two guava shrubs, one being *Psidium guayaba* and the other one *Feijoa sellowiana* both within the same climatic zone, 4 km away from locality 1.

2.1.3. Locality 3 (L3) is situated 1800 km away from the former and it is about 1000 mts. over sea level, in Yuto (lat. 24,5°; long.64,5°), Jujuy province. It is a region of tropical climate in the Northwestern of Argentina.

2.1.4. Locality 4 (L4) corresponds to Montecarlo (lat. 26,5°; long. 55°), Misiones province (Northeastern region), in the Mesopotamia. It is 1200 km from locality 1, and it presents sub-tropical climate.

The analysis of the present work is divided into two sections. Experiments 1, 2, 3 and 4 refer to studies on fruits of guava shrubs (known as *Feijoa sellowiana* or false guava of green and little juicy fruits) and the true guava *Psidium guayaba* which produces yellow and very juicy fruits. The second section in the work, that is, experiments 5 & 6 are based on the study of other fruit trees.

2.2.1. Experiment 1 (E1) was performed on the whole production of a false guava shrub which could be *Feijoa sellowiana*, from locality 1 during the period March-April 1992; this comprised practically all the fruiting of the year. In order to verify if the nutrition received from the harvested fruits was enough for the complete development of the parasites within, research was carried out in the following way: a sample of 100 fruits was put with the pulp placed on larval food [28] and another similar sample was placed on vermiculite.

Isolated fruits of one day sampling were classified into group A (14 to 40 mm) n=40 and group B (larger than 40 mm) n=56. In this experiment, material was examined daily; the recovery of pupae began on the sixth day after arrival of fruits to the laboratory, and it was repeated on the eighth, the thirteenth and the twentieth days. Afterwards no pupae were recovered.

2.2.2. Experiment 2 (E2) was carried out in the following season (1993) on fruits of the same shrub, sampling its whole production. The same design was repeated and three groups were visually separated: A' the smallest fruits, B' the intermediate ones and C' the largest ones.

2.2.3. Experiment 3 (E3) was performed at the same time as experiment 2, but it was taken in locality 2, at approximately 4 km from locality 1. The situation was different because in this place, two guava shrubs were nearby, separated by a few centimeters from each other: *Feijoa sellowiana*, which had been produced many years ago by a shoot of that used in the previous experiment and one *Psidium guayaba* . In this situation, flies had the possibility of selecting between two different host fruits.

2.2.4. Experiment 4 (E4) was performed simultaneously with experiments 2 and 3 but coincided in date with *Psidium guayaba* sampling. Samples of *P. guayaba* were taken in similar conditions in locality 3, Yuto, Jujuy, and sent to our laboratory where they were treated in the manner described above.

2.2.5. Experiment 5 (E5) was carried out in locality 4, Montecarlo, Misiones. Samples were taken during two different periods in which the presence of *A. fraterculus* on two species of fruit-trees was demonstrated through a fly-trapping system. Sweet oranges were collected in

June and August and peaches during November of the same year in three sampling periods 25 days long in which 100 fruits of each were isolated.

2.2.6. Experiment 6 (E6) was performed in locality 1 which contains shrubs species with different periods of fructification during the year. Different fruits were isolated, as previously described, to study the infestation behaviour.

2.3. Chromosomal analysis

The chromosomal analysis was performed on materials from the four localities using the techniques described in Lifschitz et al. [17].

3. RESULTS AND DISCUSSION

3.1. Experiment 1

95% of fruits placed on additional food were infested, and 99% of fruits without additional food were not significantly different in the distribution and recovery of pupae, analyzed with the Wilcoxon method for paired data. These results were taken as a reference, and,in the remaining trials no additional food was used.

The analysis of fruits classified by size (Figure 1) shows differences in the mean number of recovered pupae, in the mode (expressed in number of recovered pupae per fruit) and in the maximum number of recovered pupae being associated with fruit size. The difference among means was highly significant and their variances were equal (Table I).
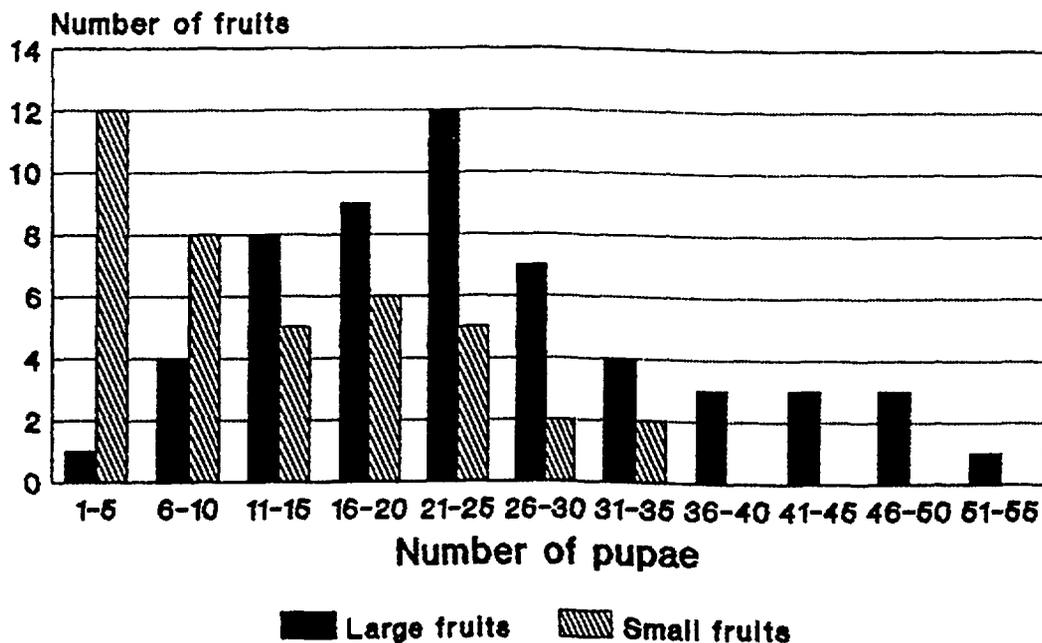
Number of fruits

Fig. 1. number of pupae recovered per fruit classified in two sizes.

TABLE I.   MEAN AND VARIANCE ANALYSIS OF THE DISTRIBUTION OF
RECOVERED PUPAE /FRUIT, DISCRIMINATED BY SIZE: A (15 TO
40mm) AND B (>40mm). * (E1,L1)

Group	% Inf.	X pupae/fruit	σ^2 (t2)	Mo	Maximun nunber of pupae
A	99	12,35 ⎤ *	97,83 ⎤ ns	9	35
B	99	24,4 ⎦	136,94 ⎦	25	55

* Mo Mode
%Inf % of infested fruits.

The study about recovery of pupae during different days in relation with fruit size is shown in Figure 2. A mode for small fruit can be detected on the eighth day and for large fruit on the twentieth day. Then a dichotomous design was planned to understand if these recoveries were the result of different oviposition behaviours, in which the day of recovery was considered as positive (Positive situation= collection date= oviposition event) and the rest as negative for both groups. Table II shows this analysis as well as the percentage distribution for different situations concerning both groups.

The maximum frequency for group A includes the first three collection dates, and for group B the four positive signs. In order to manage only the positive situations, they were regrouped without taking into account the array (Table III).

Fig. 2. Proportion of pupae recovered along time from guava fruit collected at Castelar.

TABLE II.   DICHOTOMOUS ANALYSIS OF PUPAE COLLECTION DAYS. (E1,L1)

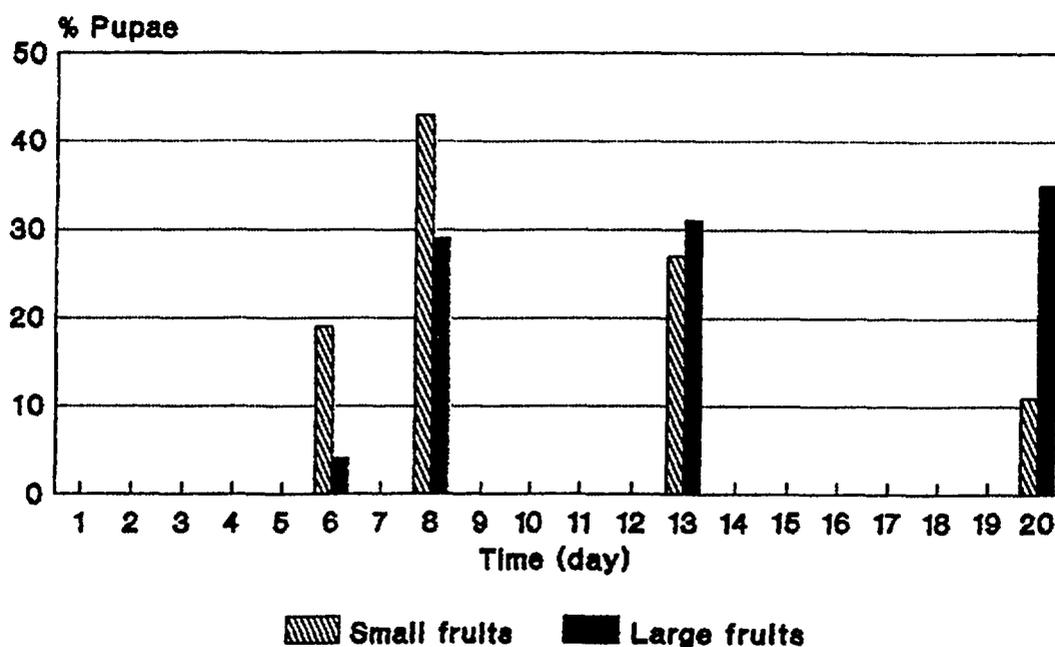| A %	B %	Collection days			
		6	8	13	20
33,3	20	+	+	+	0
11,1	16	0	+	+	0
11,1	0	+	+	0	0
5,6	0	0	+	0	+
16,7	0	0	0	+	0
5,6	0	+	0	+	0
2,8	0	0	0	0	+
8,3	29	+	+	+	+
2,8	27,3	0	+	+	+
2,8	3,6	0	0	+	+
0	1,8	+	+	0	+
0	1,8	+	0	+	+

+ Pupae presence
0 Pupae absence

80% of group B data corresponds to 3 and 4 positive situations and no case of 1 positive is reported. 72% of group A cases corresponds to 2 and 3 positive situations. These results indicate that oviposition behaviour, measured as the number of pupae and the moment in which they were obtained, is different according to the size of host fruits.

TABLE III.   SUMMARY OF POSITIVE SITUATIONS FROM TABLE II. (E1,L1)

	A %	B %
4+	8,3	29
3+	36,1	50,7
2+	36,2	19,6
1+	19,5	0

If the sampling based on the number of recovered pupae allows us to distinguish behaviours indicating that the fly selects the fruit at the moment of oviposition, a hypothesis could be postulated: females prefer to oviposit into the biggest fruits several times (ovipositions) and they would be more attracted by riper fruits. This is demonstrated by 64% recovery within group B on the last registered date, in comparison to 22% of group A.

Data discussed up to now do not agree with previously reported bibliographic data about oviposition autocontrol of the species via the "oviposition deterring pheromone" (ODP) which limits the presence of eggs per fruit between 1 to 5. In this sampling the ODP mechanism would not act on this host situation for this insect population.

3.2. Experiment 2

The distribution of fruit percentages with different numbers of collected pupae for groups A', B' and C' are shown in Table IV. Although an overlap of the three distributions exists, each mode is associated with fruit size. Group A' presents a mode of 15 pupae and a maximum of 50; group B' a mode of 25 and the same maximum as A' and group C' (fruits of the biggest size) a mode of 35 and a maximum of 105 pupae (Table IV).

Two years data are congruent in that 1) a mechanism, associated with fruit size, exists, allowing the female to select the oviposition site and 2) the amount of recovered pupae in these fruits does not agree with the pheromonal autocontrol mechanism previously suggested.

TABLE IV.   SUMMARY OF DATA FROM GRAPHIC III. (E2,L1)

Group	%Infest	Mo	R
A'	95	15	50
B'	89	25	50
C'	92	35	105

3.3. Experiment 3

In this situation the true guava began to ripen earlier than its neighbour, consequently two samplings could be carried out, ten days separating one from each other. This allowed to take samples of both shrubs simultaneously.

Results show that there is only a trend of the mode to move towards higher values during the second sampling without alteration of the infestation rank, in comparison with data of the other shrub, showing that it would be less attractive to flies although, observing the distribution of the mode and maximum limits, the behaviour per fruit seems to be similar (Table V).

TABLE V.   SUMMARY OF ATTACK BEHAVIOUR TO GUAVA FRUITS. (E1,E2,E3,E4, L1,L2,L3)

			Green Fruits			Yellow Fruits		
			%infes	Mo	Max. range	% infes.	Mo	Max. range
Locality 1	Exper.1(April 92)	small fr.	99%	9	35			
		large fr.	99%	23	55			
	Exper.2(April 93)	small fr.	95%	15	50			
		medium fr.	89%	25	50			
		large fr.	92%	35	105			
Locality 2	1 st Sampling	Exper.3 (april 93)	45%	5	25	85%	5	35
	2nd Sampling					95%	5	25
Locality 3	Exper.4(March 93)					83%	1-5.	156
						42%	1-10.	45

3.4. Experiment 4

Results of two samplings carried out within the same area but separated from one another by three days are very different (Figure 3). The first shipping of fruits showed a greater attack and a curve moving towards the maximum production of pupae. Differences are so evident that the data seem to be giving information about attacks produced by physiologically different specimens of this pest.

The attack behaviour of *A. fraterculus* on guava fruits, measured through the number of pupae, varies according to the different ecological situations presented in this work and is summarized in Table V. In a general manner it is observed that: 1) the autocontrol via ODP seems not to be total; 2) a strong correlation exists between fruit size and quantity of pupae recovered from them; 3) yellow fruits are more attractive than green ones when choice is possible, although behaviour for the first two parameters is invariant. However, it seems that in Yuto ecological situation, both curves are different.

3.5. Experiment 5

The study performed on oranges from locality 4 lasted 25 days, sufficient time to complete it. The third shipping contained a hundred fruits, but no larvae were found. In the samples of peaches from locality 4, the presence of larvae could be detected while examining the arriving fruits. One hundred fruits of each sample were isolated, the infestation results were 50%, 40% and 68%, the mode was 1 pupa per fruit and the maximum numbers were 12, 16 and 8 pupae, respectively. These results are completely different from those observed on guava fruits (Table VI).
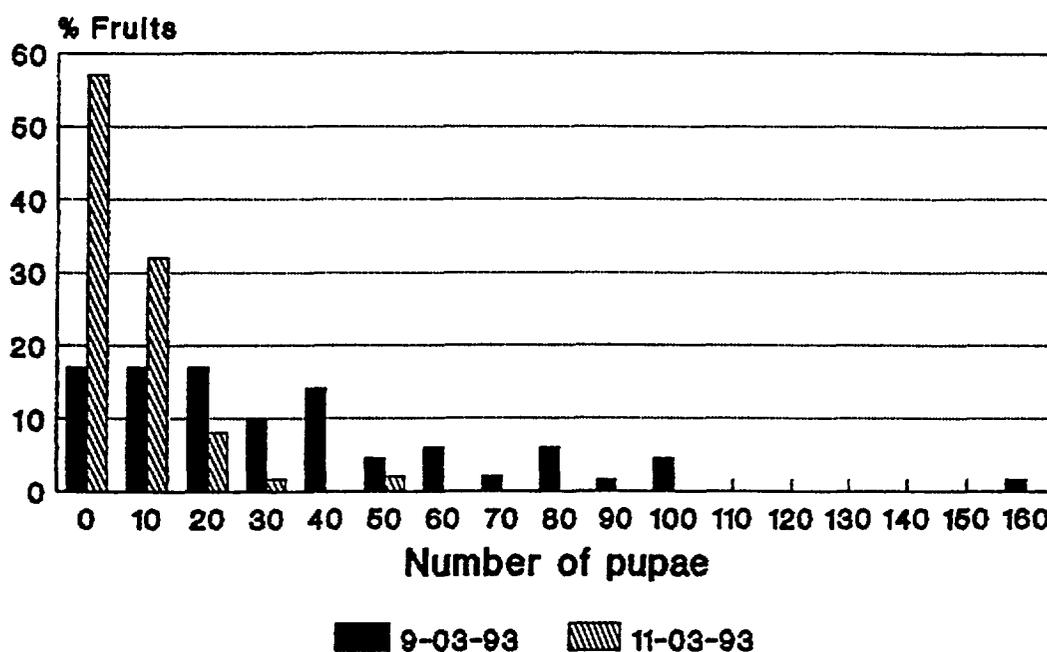

Fig. 3. Proportion of pupae recovered per fruit (two samples from Yuto Jujuy).

Results of experiment 5 indicate that, although during these samplings *A. fraterculus* was present, a host fruit was not always chosen. A sampling of other hosts of this fly should be performed during its presence in the area. However, the most evident result is that oranges were not chosen for oviposition; instead peaches were, and oviposition behaviour (Figure 4) is different than that observed on previously described guava samples.

TABLE VI.   SUMMARY OF ATTACK BEHAVIOUR TOWARD PEACHES. (E5,L4)

	%Inf	Mo	Range
1st.	50	1	12
2nd.	40	1	16
3rd.	68	1	8

3.6. Experiment 6

This experiment was carried out in locality 1 during the period from the first to second crop of guava fruits (experiments 1 & 2), which is approximately 12 months. The purpose was not only to identify hosts of the species but also to study its behaviour during this lapse.

Figure 5 shows the percentage of infestation in different species and varieties. During the lapse from May to September, no hosts of the pest were detected within this area. Two situations could be distinguished within the sampled material: 1) damsons, guava and sour-fruits, which were only attacked by *Anastrepha* although, due to the decay condition of the sours, the analysis of the pest distribution was not possible; and 2) peaches and pears, which were hosts of both *Ceratitis capitata* and *Anastrepha*. To avoid confusion, the study of distribution within these last two fruit species was not performed.   Instead, only the percentages of attacks in fruits were analyzed (Table VII).
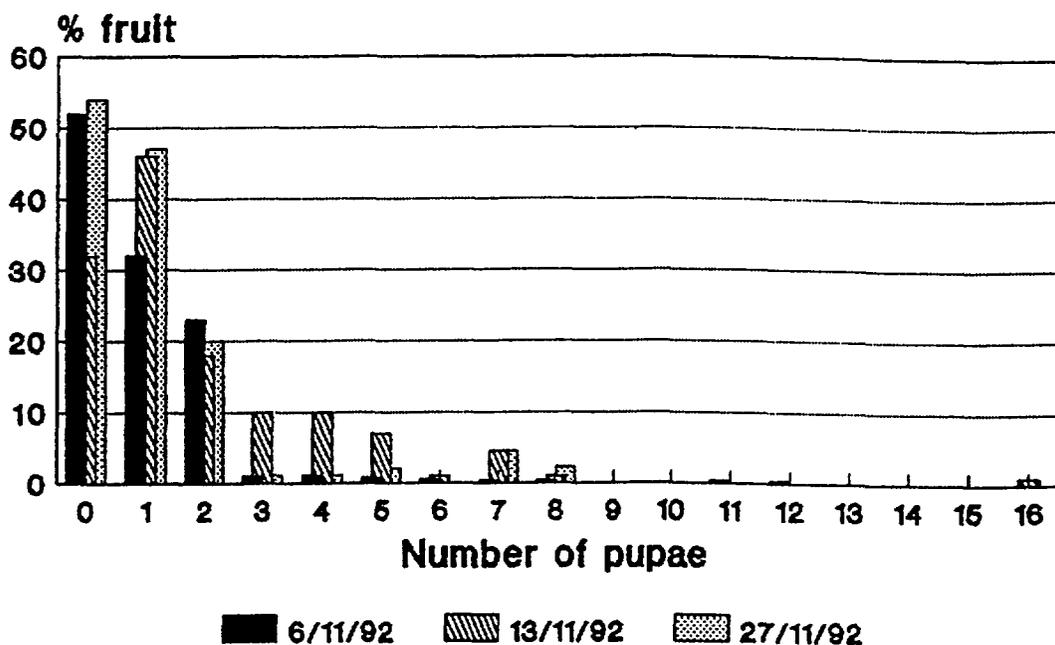
Fig. 4. Proportion of pupae recovered per fruit (three samples of peaches from Misiones).

% Infestation

1992

with A. fraterculus and C. capitata
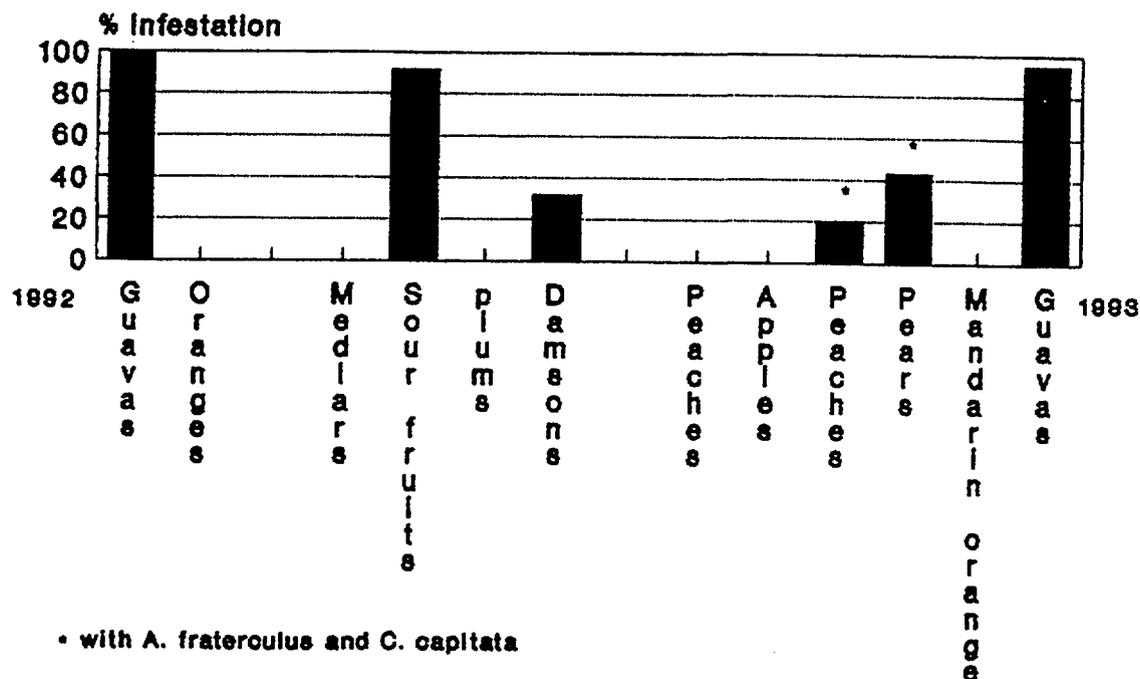
Fig. 5. Infestation of fruit from locality 1 during one year.

TABLE VII.   ANALYSIS OF FRUITS INFESTED BY *CERATITIS CAPITATA* AND *Anastrepha fraterculus*. (E6, L1)

	Peaches	Pears
Infestation	87%	43%
A. fraterculus only	26%	---
C. capitata only	38%	83%
A. fraterculus & *C. capitata*	36%	17%

3.7. Chromosomal analysis

As described previously [17], *fraterculus* Arg.1 karyotype was present at a high rate in all geographical populations. Nevertheless, a great amount of chromosomal variation was detected during the present cytological samplings within populations. Polymorphisms for the X chromosome, Y chromosome, autosomal pair 2 and presence of B chromosomes free or attached to some autosome of the complement were observed.

With respect to the X chromosome, five variants could be found: the acrocentric X_1 of *fArg 1*; an acrocentric X_2, longer than the X_1 [17]; a submetacentric X chromosome (X_3) in which the short arm is half the long arm (Picture 1); a submetacentric one which is almost metacentric (X_4) (Picture 2) and a subtelocentric one named X_5 (Picture 3).

Four morphological variants concerning the Y chromosome were detected: the small submetacentric Y_1 of *fArg 1*; a subtelocentric Y chromosome (Y_2) (Picture.2); a telocentric

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one (Y$_3$) (Picture 4) and an acrocentric one (Y$_4$) (Picture 5). Autosomal pair 2, which can show 1) heteromorphism (2h) (Picture 6), 2) a satellite on one or both members of the pair (2s), or unequal size (2*) (Pictures 4 & 8). A similar situation can be found on other autosomes (Ah, As or A*) (Pictures 2 & 7).

Further variation in size, morphology or number of chromosomes, concerns the presence of B chromosomes. Sexual aneuploids were also detected (Picture 8).
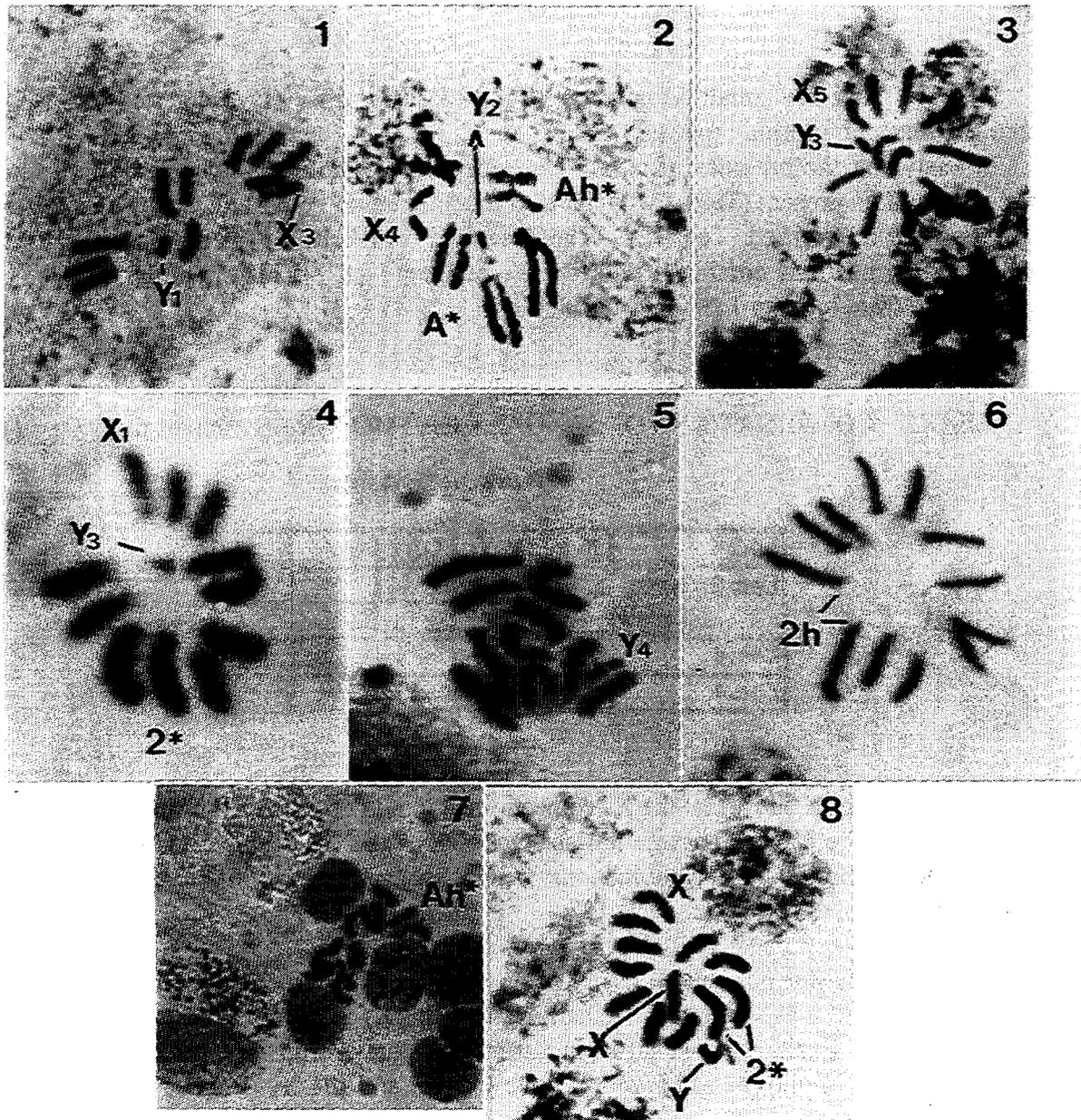
Data in table VIII are representative of the variability relative to the number of cytologically analyzed individuals.

TABLE VIII. CHROMOSOMAL VARIANTS PER LOCALITY AND HOST FRUIT.

Cytological Variation

Locality	f Arg1: $X_1 Y_1$ frequency/population	X_2	X_3	X_4	X_5	Y_2	Y_3	Y_4	2s	2h	2*	As	Ah	A**	B
1,-Green Guava	38% (n= 52)		X	X			X		X	X	X	X			X
2,- Green Guava Yellow Guava				X		X					X		X	X	
	50% (n= 56)				X		X			X		X				X
3,- Yellow Guava	71% (n= 35)					X	X	X					X	X	X
4,- Peaches	38% (n= 48)	X				X	X	X		X	X				X

Despite relatively small samples, the chromosome diversity suggests that we are studying a very polymorphic species.

In analyzing the presence of sexual chromosomes within each locality, not only more than one variant is present (Table VIII) but also recombination among individuals of different karyotypes can be verified (Table IX). The described X_3 (Picture 1) is similar to that of *A. sororcula* [14], but it is combined with the Y_1 of *fArg 1* [17]. This situation was detected among larvae from locality 1 (Picture 1, Table IX).

TABLE IX.   COMBINATIONS OF SEXUAL CHROMOSOMES, (appart from *fArg 1*).

Karyotypes Found

Loc. 1	$X_1 X_4$	$X_1 Y_3$	$X_4 Y_3$	$X_3 Y_1$	
Loc. 2 Green Yellow	$X_4 Y_2$				
		$X_1 X_5$	$X_5 Y_3$	$X_1 Y_3$	
Loc. 3	$X_1 Y_3$	$X_1 Y_4$			
Loc. 4	$X_2 X_2$	$X_1 X_2$	$X_1 Y_2$	$X_1 Y_3$	$X_1 Y_4$

Data in Table IX show that the X_1 comes joined to all described Y chromosome variants as well as to all the X chromosome variants except for the X_3 (Table IX). Besides, other different sexual chromosome combinations within polymorphic populations were detected (Tables VIII, IX).

Although it is not possible to establish an association either with host species or with geographical origin, it is worthwhile remarking that the X_2 [17] was only found in peaches from Montecarlo. Besides, when different varieties of guava fruits are analyzed separately, the X_5 (Picture 3) is only detected within yellow fruits and the X_4 (Picture 2) within green fruits (Table VIII).

Assuming that the frequency of each variant can be different within each population, in most of the cases in which different sexual chromosome combinations occur, no isolating processes are acting among individuals carrying them. These data suggest that not all chromosomal polymorphisms mark reproductive barriers.

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However, two possibilities should be taken into account: **1)** our analyses are just pointing out sibling species as proposed for Brazilian populations [13] or **2)** we have two simultaneous phenomena: **A)** some situations would be chromosome polymorphisms such as those found within Argentine populations of *Ceratitis capitata* [29, 30], **B)** some others could be representing sibling species.

It would be essential to analyze what effect these karyotypic markers might represent as reproductive barriers. To date, despite the distinctive behaviour detected in guava fruits versus peaches, our knowledge does not allow us to talk about an isolation caused by the karyotype. However the X_2 chromosome described for Montecarlo, Misiones, where peaches were sampled, could be one of the causes for such differential behaviour.

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LA MOSCA SURAMERICANA DE LAS FRUTAS,
Anastrepha fraterculus (DIPTERA: TEPHRITIDAE)
EN COLOMBIA

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Abstract-Resúmen

THE SOUTH AMERICAN FRUIT FLY, *Anastrepha fraterculus* (DIPTERA: TEPHRITIDAE) IN COLOMBIA.
    Anastrepha fraterculus (Wied) is the most important fruit fly in Colombia. It has been trapped from the sea level up to 2000 m of altitude, but it is more abundant in the coffee growing area located at 1300 to 1700 masl, with temperatures between 18 to 22°C (_min 11°C, _max 25°C). The main host in that area is *Coffea arabica* L., but it also has 14 additional identified hosts that belong to 9 families. In the hot climates from 0 to 1000 m of altitude it breeds in mango (*Mangifera indica* L.) and guava (*Psidium guayava* L:) The pest has not been stabilised in the cultivated upper lands betwen 2300-2600 masl.

LA MOSCA SURAMERICANA DE LAS FRUTAS, *Anastrepha fraterculus* (DIPTERA: TEPHRITIDAE) EN COLOMBIA.
    Anastrepha fraterculus (Wied) es la mosca de las frutas de mayor importancia en Colombia, se distribuye desde el nivel del mar hasta 2000 metros de altitud, pero es especialmente abundante en la zona cafetera en donde crece la mayoría de las plantas hospedantes identificadas. La zona más favorable para el desarrollo de la plaga corresponde al clima medio o templado entre 1300 y 1700 msnm con temperatura entre 18 y 22°C (_min 11°C, _max 25°C). El principal hospedante es el café (*Coffea arabica* L), pero se desarrolla también en 14 especies de plantas cultivadas o silvestres, pertenecientes a 9 familias. En las zonas cálidas (0 a 1000 msnm). Los hospedantes identificados son mango (*Mangifera indica L.*) y guayaba (*Psidium guayava L.*). en las zonas frías (2300 a 2600 msnm) apta para frutales y hortalizas varias, la plaga no se ha establecido.

1. INTRODUCCION

Colombia tiene una ubicación estratégica en el trópico (Latitud 4°, 18', 30" y 12°, 30' 40" N; Longitud 66° 51 y 79° W) y una gran variedad de climas y suelos apropiados para el cultivo de frutales nativos o introducidos. Este factor, junto con la intensificación del consumo interno y de los estudios de factibilidad favorables a la aceptación de varias frutas y hortalizas en los mercados internacionales han incentivado el cultivo tecnificado de varias frutas y hortalizas. Las limitantes para la producción y comercialización de estos productos son de diversa índole y uno de los más importantes lo constituye el daño de moscas de las frutas de la familia Tephritidae. Entre la diversidad de especies nativas de este grupo, identificadas en el país, la mosca suramericana de las frutas (M.S.A.F.) *Anastrepha fraterculus* Wied. es la más importante por su amplia distribución y adaptación a mayor diversidad de plantas hospedantes. A pesar de esto, no se ha hecho una cuantificación de los daños directos pero junto con la mosca del mediterráneo son el principal limitante cuarentenario para la exportación.

2. MATERIALES Y MÉTODOS

Los datos que se presentan en esta publicación corresponden especialmente a resultados de muestreos y trampeos realizados en desarrollo de dos proyectos financiados por la FAO: el proyecto andino de reconocimiento de moscas de las frutas realizado entre 1971 y 1974 (Martín 1973) y el PTC/COL/4505 entre 1986 - 1988, para el reconocimiento de las moscas de las frutas y prevención de la mosca del mediterráneo (Olalquiaga 1987, FAO 1988). Adicionalmente se incluyen resultados de las campañas nacionales bajo responsabilidad del Instituto Colombiano Agropecuario ICA y trabajo de tesis relacionados con estas campañas.

Para determinar las plantas hospedantes se han recolectado muestras de frutas de zonas de climas frío, templado y cálido mantenidas en cajas de cría hechas en madera o de materiales sintéticos, de las cuales se obtuvieron larvas y pupas que se pasaron a tierra orgánica o a vermiculita húmeda hasta la emergencia de adultos, estos han sido, identificados por entomólogos del SEL (USDA) o taxónomos como Ch. Korytkowski, y V. Fernández de Panamá y México o se han determinado por entomólogos colombianos. Parte de los resultados han sido publicados y otros se encuentran en informes y tesis de estudiantes no publicadas.

3. RESULTADOS Y DISCUSIÓN

3.1. Distribución

Anastrepha fraterculus se captura desde el nivel del mar hasta 2000 metros de altitud en la zona dedicada a la agricultura. Esta área se localiza en las laderas de las tres cordilleras y en los valles interandinos que se extiende desde el límite con el Ecuador hasta Venezuela; esta zona se ha desarrollado paralelamente con el cultivo del café (*Coffea arabica* L.). No se ha confirmado el establecimiento de esta plaga en frutales de clima frío, que crecen en las altillanuras localizadas entre 2300 y 2400 msnm en la región central de la Cordillera oriental (departamentos de Boyacá y Cundinamarca) y en el sur del país (departamentos de Nariño y Putumayo), y no se tiene información del estado de la plaga de las Llanuras orientales.

Las condiciones climáticas de los estratos altitudinales y los frutales predominantes en las zonas agrícolas se presentan en la Tabla 1 (Federación Nacional de Cafeteros 1979). En la Tabla 2 se presenta la clasificación taxonómica de las especies frutícolas incluidas.

Aún cuando la distribución de *A. fraterculus* es amplia, la población de adultos es más abundante en la zona cafetera óptima, disminuye en las zonas marginales alta y baja y en la zona cálida, y no se ha confirmado la presencia de adultos ni larvas en las zonas frías por encima de 2000 msnm.

Las temperaturas más favorables para la adaptación de la especie están entre 18 y 22°C, con promedio mínimo de 11°C y máximo de 25°C. Probablemente la limitante para la adaptación en las sabanas altas es la temperatura en donde se tiene un promedio de 13°C. En estas zonas se registran temperaturas promedio mínimo de 6°C y máximo de 20°C, con temperaturas entre -3 y -6 grados centígrados en las noches.

TABLA 1. CARACTERÍSTICAS DE LAS ZONAS AGRÍCOLAS DE COLOMBIA Y FRUTALES PREDOMINANTES

Denominación	Altitud msnm	Temperatura °C		HR %	Precipitación mm/año	Frutales Predominantes
		Min	Max			
Fría	2200-2500	6°	19°	70-80	600-700	MYRTACEAE: Freijoa ROSACEAE: Fresa, manzana, ciruela, durazno, pera, mora, frambuesa. SOLANACEAE: Uchuva
Cafetera marginal alta	1800-2000	15°	18°	70-80	1700-1800	LAURACEAE: Aguacate MIRTACEAE: Freijoa, guayaba dulce*, arrayán* MORACEAE: Brevo ROSACEAE: Fresa, manzana, mora* RUBIACEAE: Café* RUTACEAE: Naranja dulce, naranja agria[1]* SOLANACEAE: Tomate de árbol*., lulo* PASSIFLORACEAE: Granadilla, curuba*

* Hospedante de *A. fraterculus* Wied.

TABLA 1 (cont.)

Denominación	Altitud msnm	Temperatura °C		HR %	Precipitación mm/año	Frutales Predominantes
		Min	Max			
Cafetera Optima	1300-1700	18°	21°	70-80	2200-2600	ANNONACEAE:Anón colorado, chirimoya*
						CACTACEAE: Pitahaya*, higo
						LAURACEAE: Aguacate
						MYRTACEACE: Guayaba dulce*
						RUBIACEAE: Naranja dulce, naranja agria[3]*. tangelo
						ROSACEAE: Níspero japonés*
Cafetera marginal baja	1000-1300	210°	240°	70-80	1000-2000	ANNONACEAE:Anón de azúcar, chirimoya*, gunábana
						BOMBACACEAE: Zapote de los Andes
						CACTACEAE: Pitahaya*
						CARICACEAE: Papaya
						LAURACEAE: Aguacate
						MYRTACEACE: Guayaba dulce*
						RUBIACEAE: Café
						RUTACEAE: Naranja dulce*, naranja agria*. mandarina
						ROSACEAE: Níspero japonés*

* Hospedante de *A. fraterculus* Wied.

TABLA 1 (cont.)

Denominación	Altitud msnm	Temperatura °C Min	Max	HR %	Precipitación mm/año	Frutales Predominantes
Cálida	0-1000	250°	350°	70-80	1000-2000	ANACARDIACEAE: Mango[4]*, ciruelo jobo
						ANNONACEAE: Guanábana
						BROMELIACEAE: Piña
						CARICACEAE: Papaya
						CUCURBITACEAE: Melón, sandía
						GUTIFERACEAE: Mamey, mangostino
						LAURACEAE: Aguacate
						MYRTACEACE: Guayaba dulce*
						PASSIFLORACEAE: Badea, maracuyá
						RUTACEAE: Limón, toronja
						SAPOTACEAE: Mamey (sapote) colorado, níspero

* Hospedante de *A. fraterculus* Wied.

TABLA 2. Clasificación de especies de frutales cultivados en Colombia

Familia	Nombre Común	Nombre Científico
ANACARDIACEAE	Mango	*Mangifera indica* L.
	Ciruelo jobo	*Spondias sp.*
ANONACEAE	Anón colorado	*Anona reticulata L.*
	Anón de azúcar	*A. squamosa L.*
	Chirimoya	*A. chirimolia Mill**
BOMBACACEAE	Guanábana	*A. reticulata L.*
BROMELIACEAE	Zapote de los Andes	*Quaribea (Matisia) cordata H.B.K.*
	Piña	*Ananas comosus Magness*
CACTACEAE	Pitahaya	*Acanthocereus pitahaya Dugand**
	Higo o tuna	*Opuntia ficus carica L.*
CARICACEAE	Papaya	*Carica papaya L.*
CUCURBITACEAE	Patilla o sandía	*Citrulus vulgaris Schrad*
	Melón	*Cucumis melo L.*
GUTIFERACEAE	Mamey	*Mamea americana L.*
	Mangostino	*Garcinia mangostana L.*
LAURACEAE	Aguacate	*Persea americana L.*
MYRTACEAE	Pomarrosa	*Eugenia jambos L.*
	Arrayán	*Mytus foliosa H.B.K.*
	Guayaba dulce	*Psidium guayava L.*
	Feijoa	*Feijoa sellowiana Berg.*
MORACEAE	Brevo	*Ficus carica L.*
PASIFLORACEAE	Granadilla	*Passiflora ligularis Juss*
	Curuba de castilla	*P. mollisima Bailey*
	Badea	*P. guandrangularis L.*
	Maracuyá	*P. edulis f. flaricarpa DeGener*
ROSACEAE	Níspero japonés	*Eriobotrya japonica Lind.*
	Fresa	*Pragaria sp*
	Manzana	*Malus sp*
	Ciruela	*Prunus doméstica L.*
	Durazno	*Prunus persica Stokes et Zucc*
	Pera	*Pyrus communis L.*
	Mora	*Rubus glaucus*
	Frambuesa	*Rubus sp.*
RUBIACEAE	Café	*Coffea arabica L.*
RUTACEAE	Mandarina	*Citrus reticulata Blanco*
	Naranja agria	*C. aurantium L.*
	Naranja dulce	*C. sinensis Osbeek*
	Limón	*C. limón L.*
	Toronja	*C. paradisi Macf.*
SAPOTACEAE	Mamey (Zapote) colorado	*Colocarpum mammosum L.*
	Níspero	*Achiras sapota L.*
SOLANACEAE	Lulo	*Solanum quitoense Lam.*
	Tomate de árbol	*Cyphomandra betacea Sendt.*
	Uchuva	*Physalis peruviana L.*

3.2. Hospedantes

El café es el principal hospedante de *A. fraterculus*, cultivo que fructifica durante todo el año, lo cual permite el desarrollo continuo de la plaga. Las larvas se desarrollan en los frutos maduros sin causar caída de estos. Adicionalmente se han determinado como hospedantes 14 especies de frutales taxonómicamente distribuidas en 9 familias y que crecen en la zona cafetera (Tabla 3).

De estas algunas se cultivan en asocio con café o cerca al café como es el caso de *Rubus glaucus, Citrus sinensis, Anona squamosa, A. chirimolia, Acanthocereus pitahaya, Psidum guayava, Cyphomandra betaea, Solanum quitoense*, otros crecen espontáneamente en huertos caseros o se utilizan como cercos vivos o como sombrío del café; en esta categoría se consideran *Eugenia jambos, Myrthus foliasa, P. guayava, Eriobotrya japonica, C. aurantium* (Murillo 1931, González-Mendoza 1952, Nuñez-Bueno L. 1981, Portilla et al 1994, Nuñez 1991).

TABLA 3.   Hospedantes de *A.fraterculus* (Wied) confirmados en Colombia hasta 1996.

Familia	Especie: Nombre científico	Nombre Común
ANACARDIACEAE	*Mangifera indica* L.	Mango
ANONACEAE	*Anona squamosa* L.	Anón
	Anona chirimolia M.d'Arvovie	Chirimoya
CACTACEAE	*Aconthocereus pitahaya* Dugond	Pitahaya
	(=*Selenicereus megalanthus*)	
	(=*Cereus triangularis* Han)	
	(=*Hylocereus sp.*)	
MYRTACEAE	*Eugenia jambos* L.	Pomarrosa
	Myrthus foliosa H.B.K	Arrayan
	Psidium guayava L.	Guayaba
PASSIFLORACEAE	*Passiflora mollisima* Ldl	Curuba
ROSACEAE	*Eriobothrya japonica* Ldl	Níspero Japonés
RUBIACEAE	*Coffea arabica* L.	Café
RUTACEAE	*Citrus aurantium* L.	Naranja agria
	Citrus sinensis Osbeck	Naranja dulce
SOLANACEAE	*Cyphomandra betaceae* Sendt	Tomate de árbol
	Solanum quitoense Lam.	Lulo

1991, Nuñez-Bueno 1996; Díaz y Vásquez 1993).

El mango (*Mangifera indica*) es hospedante esporádico de la M.S.A.F. cuando crece en cultivos establecidos entre 500 y 700 msnm o cuando crece en huertos caseros o cercos en la zona marginal cafetera baja, pero no se ha confirmado en cultivos de la zona cálida. En todos los estratos altitudinales este frutal es hospedante primario de A. obliqua (McQuart). (Observación personal)

La guayaba (*P. guayava*) crece espontáneamente desde el nivel del mar hasta 2000 metros de altitud y se han establecido huertos tecnificados en la zona cálida. En toda el área de distribución el cultivo es atacado por *A. striata*.

La zona cálida por debajo de 1000 metros es atacada por A. striata, A. fraterculus y A. obliqua. En la zona cafetera marginal baja y óptima es atacada simultáneamente por A. striata y por A. fraterculus, en la zona cafetera marginal alta entre 1700 y 1920 metros se presenta adicionalmente A. ornata (Schiner). (Luna 1973, Nuñez

El número de adultos de A. fraterculus en trampas colocadas en cítricos dentro de la zona cafetera es abundante durante todo el año, se ha observado daño en C. sinensis y C. aurantium en zonas citrícolas no tecnificadas y establecidas hace 30 ó 40 años (Martín 1973, Nuñez-Bueno 1981, Nuñez 1991). En áreas nuevas localizadas en la zona cafetera central, sembradas con tangelo y mandarina, así como en las zonas cálidas en donde se cultiva limón y toronja no se ha comprobado el daño.

La importancia económica de esta especie contrasta con la escasez de estudios en el país. Norrbom y Kim (1988) relacionan 90 hospederos en el área de distribución de A. fraterculus desde el sur de los Estados Unidos hasta el sur de Argentina. Es necesario que se de más importancia a la investigación que contribuya a definir la relación con hospederos silvestres y cultivos y estudios de citogenética que permitan esclarecer el status taxonómico del complejo A. fraterculus y de la gran diversidad de especies del género Anastrepha. Estos estudios son prioritarios para el lineamiento y ejecución de medidas de control.

Teniendo en cuenta que las zonas frías cultivadas están libres de moscas de las frutas es necesario que se continúen y refuercen los programas de vigilancia y cuarentena para proteger la producción de frutas y hortalizas ya establecidos o de nuevos que puedan adoptarse en cultivos a campo abierto o bajo invernadero. Una medida que puede evaluarse es la liberación de insectos estériles en áreas estratégicas con condiciones favorables a los adultos para prevenir la dispersión de las poblaciones naturales.

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DETERMINACION DE DOSIS DE RADIACION GAMMA COMO TRATAMIENTO POST-COSECHA EN MANGOS INFESTADOS CON MOSCA SUDAMERICANA DE LA FRUTA

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DETERMINATION OF GAMMA RADIATION DOSE AS A POST-HARVEST TREATMENT IN MANGOS INFESTED WITH THE SOUTH AMERICAN FRUIT FLY.
Efforts are being made to determine a gamma radiation dose for mortality of third-instar larvae of *Anastrepha fraterculus* which infest mangos of the Haden variety of 400 g weight. Four radiation treatments were tested: 0.4 kGy, 0.6 kGy, 0.8 kGy and 1.0 kGy.
Using as a criterium for mortality the interruption of the biological cycle between larva and pupa, the following results were achieved: 49.61%, 63.33%, 74.86% and 90.72%. The percentages obtained have been corrected using the Abbot formula. When the criterium was based on no adult emergence, 100% mortality was achieved for the four treatments.

DETERMINACION DE DOSIS DE RADIACION GAMMA COMO TRATAMIENTO POST-COSECHA EN MANGOS INFESTADOS CON MOSCA SUDAMERICANA DE LA FRUTA.
Se busca encontrar dosis de radiación gamma para causar mortalidad en larvas del tercer estadío de *Anastrepha fraterculus* infestantes de mangos de la variedad Haden de 400 g de peso. Se ensayaron cuatro tratamientos de radiación : 0.4 kGy, 0.6 kGy, 0.8 kGy y 1.0 kGy.
Basando como criterio de mortalidad la interrupción del ciclo biológico entre larva y pupa se muestran los resultados siguientes : 49.61%, 63.33%, 74.86% y 90.72%. Los porcentajes obtenidos han sido corregidos mediante la fórmula de Abbot. Cuando el criterio se basó en la no emergencia de adultos se consiguió un 100 % de mortalidad para los cuatro tratamientos.

1. INTRODUCCION

Existen diferentes alternativas de tratamiento post-cosecha para poder exportar fruta fresca hacia mercados que imponen restricciones cuarentenarias debido a problemas fitosanitarios.

Nuestro país actualmente exporta mangos hacia mercados importantes, tratándolos con agua caliente. Sin embargo es necesario buscar nuevas alternativas siendo la radiación gamma o tecnología pico-onda una de ellas, la cual asegura una completa desinfestación sin alterar la calidad del producto tratado.

En nuestro país, Alama (1990) irradió mangos de la variedad Haden infestados con la mosca del mediterráneo, determinando dosis entre 0.75 KGy y 1.0 KGy como las más adecuadas para causar mortalidad sin alterar las características organolèpticas del producto.

2. OBJETIVO

Determinar dosis de radiación gamma que cause mortalidad de larvas del tercer estadío de la mosca sudamericana de la fruta *Anastrepha fraterculus* Wied. infestantes de mangos de la variedad Haden.

3. MATERIALES Y METODOS

Se estableció un núcleo de reproductores de la mencionada especie para ser utilizada en las infestaciones en laboratorio. Se colocaron 80 mangos en las jaulas de adultos sexualmente maduros durante 3 días.

Los tratamientos se realizaron un día antes que las larvas empezaran a abandonar los frutos. Se utilizó un irradiador gammacell 220 del Instituto Peruano de Energía Nuclear el cual tiene como radioisótopo Co-60. La distribución por tratamiento fue:

16 mangos no se irradiaron (controles)
16 mangos se irradiaron a 0.4 KGy
16 mangos se irradiaron a 0.6 KGy
16 mangos se irradiaron a 0.8 KGy
16 mangos se irradiaron a 1.0 Kgy.

Los mangos fueron colocados en cajas de recuperación y almacenados a 25°C - 28°C y 60% - 80% de humedad relativa. Las colectas de pupa se realizaron a partir del primer día post-irradiación y finalmente los frutos infestados fueron observados internamente para verificar los inmaduros muertos.

Finalmente se determinaron los porcentajes de mortalidad (se corrigieron con la fórmula de Abbot) basados en la interrupción del ciclo biológico entre larva-pupa. Los resultados se muestran en la tabla N° 1.

TABLA N° 1 : Porcentajes de mortalidad en los tratamiento con rayos gamma

TRATAMIENTOS (KGy)	MORTALIDAD (%)
0.00	17.00
0.40	49.61
0.60	63.33
0.80	74.86
1.00	90.72

Cuando el criterio se basó en la no emergencia de adultos se consiguieron mortalidades del 100%.

4. CONCLUSIONES

De acuerdo con los resultados podemos concluir:

- Cuando el criterio fue la interrupción de ciclo entre larva-pupa

1. El tratamiento a 0.4 KGy causó una mortalidad de 49.61%

2. El tratamiento a 0.6 KGy causó una mortalidad de 63.33%

3. El tratamiento a 0.8 KGy causó una mortalidad de 74.86%

4. El tratamiento a 1.0 Kgy causó una mortalidad de 90.72%.

- Cuando el criterio se basó en la no emergencia de adultos la mortalidad fue del 100%.

SPECIFIC BIBLIOGRAPHY ON *Anastrepha fraterculus*

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Abstract

SPECIFIC BIBLIOGRAPHY ON *Anastrepha fraterculus*.

Most publications on *Anastrepha fraterculus* are in the literature sources from South America, and papers were published in Spanish or Portuguese. Many literature sources mentioned in this list are not indexed in entomological abstracts and, consequently, are not easily available outside their home state or country. Due to this fact, the inclusion of a list of specific bibliography on *A. fraterculus* could be a help for researchers and students interested in fruit flies.

In this list are included only references directly dealing with *Anastrepha fraterculus*. Obviously, many more references exist that consider this species, but general or non-specific work or data concerning *A. fraterculus* were not included in the present list.

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CONCLUSIONS AND RECOMMENDATIONS

Working Group I
Taxonomy and genetics of *Anastrepha fraterculus*

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Problem:

There is a lack of knowledge of current activities among people working on SAFF. Working group meetings help to resolve this problem and promote contacts among interested parties, but more could be done.

Solutions:

(a) Establish an e-mail discussion group for the working group.

(b) Create Web pages for various aspects of the group, for example, a web page on SAFF including the bibliography that has been proposed, as well as abstracts of publications that authors can submit or that can be downloaded from the discussion group.

(c) Another possibility might be to reestablish a newsletter for the working group, although a lack of contributed articles seems to have killed these in the past.

Problem:

What are the cryptic species of the complex? How can they be diagnosed and recognized?

Solution:

We need samples preserved in various ways (depending upon the method of study to be used) and to be made available to all interested parties, from across the range of the complex and from a range of hosts in order to study the variation present, to recognize disjuctions indicating reproductive isolation, and to characterize each of the cryptic species.

Recommendation:

Develop a network of collaborators and parties conducting the taxonomic work (see recommendation on communication).

Problem:

Some methods require live material, for example, cytological studies and cross-mating experiments. Quarantine regulations may prevent the transport of live material outside of the countries of origin of different populations.

Solution:

To facilitate such studies, colonies should be established in as many countries as possible (or parts of them where multiple cryptic species are suspected), or sites should be identified where live material could be readily collected. Funding should be available for workers to travel to other colony sites to conduct comparative studies.

Recommendation:
Funding should be provided to establish colonies or promote their upkeep, and to identify reliable collecting sites.

Problem:
Because the complex is still unresolved, we won't be able to tell what published information applies to which cryptic species.

Solution:
Voucher specimens should be preserved for any studies, including ecological, behavioral, and other biological studies, so that the identity of the flies can be rechecked.

Recommendations:
(a) Authors should preserve vouchers and deposit them in a collection. Specimens should be preserved in 70% ethanol or pinned, or if possible by both methods, with complete label data.
(b) Develop a code and a data base to track samples.

Problem:
How should collaborators collect samples and how can their transport be facilitated?

Solutions:
Each worker should provide collecting and shipping protocol to be put on the Web page and circulated by other methods. Possibly international agencies could provide funds to support shipping.

Problem:
There are no workers in some areas, or it may be too big a burden on some workers to impose collecting or surveying requests upon them. Currently available samples are extremely patchy.

Solution:
Areas that need to be sampled must be indicated. This should be reevaluated as research progresses. Proposals should be prepared to fund surveys or transects in important areas. Several areas of particular interest are along a transect from northeastern to southern Brazil, and transects across the Andean countries. Surveys should be detailed and concentrate on rearing specimens from host plants.

Problem:
Results of different types of studies have not been integrated in most cases. Behavioral and ecological as well as taxonomic and genetic studies.

Solution:
Analyses of the same populations and, where possible, of the same individuals, by a variety of methods should be encouraged. Recommendation: Establish a depository for specimens similar to that at Otis Airbase (or ask APHIS to also agree to store SAFF material there).

Working Group II
Artificial Rearing of *Anastrepha* species

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Colonization of the South American fruit fly

(a) Means of oviposition

It is recommended to use the net used for *A. obliqua* (16 linen cloths/linear centimeter) covered with melted wax, and mixed with vaseline, forming a fine film.

The use of agar spheres, can be substituted with "carragenina" or "Xantam glue", to facilitate the extraction and recuperation of eggs and to reduce costs.

It is recommended to add sponges with fruit juice (preferably) inside the domes and spray sweetened water on its surface to stimulate oviposition.

(b) Adult diet

Considering that the enzymatic protein hydrolyzate with sugar (1:4) has given the best results to feed the adults of *Anastrepha* fruit flies, the use of the same formula, adding some minerals and vitamins to increase fecundity, is recommended.

(c) Adult density in oviposition cages

In the beginning, a 0.5 adults per square centimeter density is recommended. Later, an evaluation of the optimal density can be carried out.

(d) Colony cage

The standard cage for colonization (30 cm × 30 cm × 30 cm) is recommended.

The recommended net to cover the cage should be of smooth fiberglass.

(e) Larval diet

The diet ingredients should preferably be of local origin. The bulk agents are the components which usually vary in the different fruit fly diets. Inactive dried yeast (usually torula), sugar and food preservatives are the common ingredients which vary in proportion in all the larval diet formulas. It is recommended to analyse the diet formulas used for *Anastrepha ludens* and *Anastrepha obliqua* in Mexico and the USA.

It is of high priority to establish a strict program of asepsis in all the rearing rooms, materials, environment and personnel.

Mass rearing of *Anastrepha* spp.

(a) Colony

Size of the cages:
It is recommended to use the same cages used for mass rearing of *Anastrepha ludens*, *A. suspensa* and *A. obliqua*. The dimensions are indicated in the working papers presented at the workshop. It is necessary to maintain at least 90% R.H. inside the cage to maintain the optimal micro-environment.

Site for oviposition:
This should be adequate with the indicated net (16 linen clothes per linear centimeter) covered with a slight silicone film. To test net colors is recommended. Red has given good results with *A. ludens*, *A. obliqua* and *A. striata*, with a light intensity balanced to the solar spectrum.

Water in the drinking dishes:
Water should be free of minerals and with cloride for disinfestation at 0.2% A.I.

Luminosity:
A minimum of 400 lux for the starting colony stream and 2000 to 4000 lux for mass rearing is recommended.

(b) Egg collection

To homogenize the age of the eggs to synchronise egg hatch and larval/pupal age, it is recommended in order to reduce negative effects to pupae at the moment of irradiation.

(c) Quality of the different insect stages

To establish quality control parameters for each biological phase, following the recommendations indicated in the mass rearing protocols of *Anastrepha* spp. used in Mexico and the USA.

In order to maintain a competitive laboratory strain in the field, it is recommended to renew the colony with "injections" of wild material. These exercises will maintain the genetic characteristics of the laboratory strain.

Conclusions

With the experience obtained in the mass rearing of *Anastrepha ludens*, *Anastrepha obliqua* and *Anastrepha suspensa*, and the colonization of *Anastrepha serpentina*, it is concluded that the colonization, mass rearing and the possible use of the sterile insect technique (SIT) in *Anastrepha fraterculus*, is feasible.

The success of small experimental artificial rearing of *A. fraterculus* carried out in Peru, Colombia, Argentina and Brazil, also supports the possibility to mass rear this species.

The SIT requires a logical sequence for its development and application: (i) getting the fruit fly colony or strain; (ii) colonization; (iii) adaptation to mass rearing conditions and finally, (iv) application of the SIT in the field.

Knowing the genetic and/or taxonomic differences of *A. fraterculus*, it is recommended to increase research efforts (with the leadership of universities, government institutions, international organizations, etc.) to determine the strain to be produced in each country. From a practical view point, select specific colonies from damaging populations such as citrus.

To obtain the basic strain or colony, the methodology described by D. Moreno is recommended.

For the adaptation of the colonized strain to a mass rearing scale, the methodology described by J. Domínguez and collaborators and the mass rearing protocols for *Anastrepha suspensa*, *A. ludens* and *A. obliqua* is recommended.

To apply strict quality control measures from the colonization to the mass rearing are recommended, as described by M.J. Hayes.

It was recommended to all members involved in the working group to be in contact and collaborate with the plans of Perú as the leading country with specific activities to develop the mass rearing of *Anastrepha fraterculus*, in order to support their fruit fly control and eradication programme using the SIT and the other countries in the subregion.

Final recommendations

In order to obtain the strain for the colony, the methodology described by D. Moreno is recommended.

For the adaptation of a colonized strain for mass rearing, the methodology described by J. Domínguez et al. and the *Anastrepha suspensa, A. ludens* and *A. obliqua* mass rearing protocols are recommended.

To follow a strict quality control protocol since the colonization works until the mass rearing operation is recommended. The methodology described by Mary Jo Hayes for this purpose is suggested.

Working Group III
Ecology, biology, behaviour and control methods of *Anastrepha fraterculus*

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Recommendations

To carry out studies on the sexual compatibility of the different "types" of *A. fraterculus*.
To carry out studies on the efficency of control of *A. fraterculus* using parasitoids (presently mass reared). For example: *D. longicaudata* in Tapachula, Mexico.

Taking into consideration the great number of parasitoids of *A. fraterculus*, To study and develop mass rearing technique for one of these.

To define the taxonomic status of *A. fraterculus*, in the different geographic regions.

To establish research protocols for field studies and the biology of *A. fraterculus*.

To assess the economic damage of this fruit fly per regions and countries to determine where *A. fraterculus* is and where it is not a pest.

To carry out studies on the interspecific competition between *C. capitata* and *A. fraterculus*.

To develope control methods for the fruit growers with minimum use of insecticides.

To promote the use of alternate products in the region to control fruit flies including *A. fraterculus*.

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