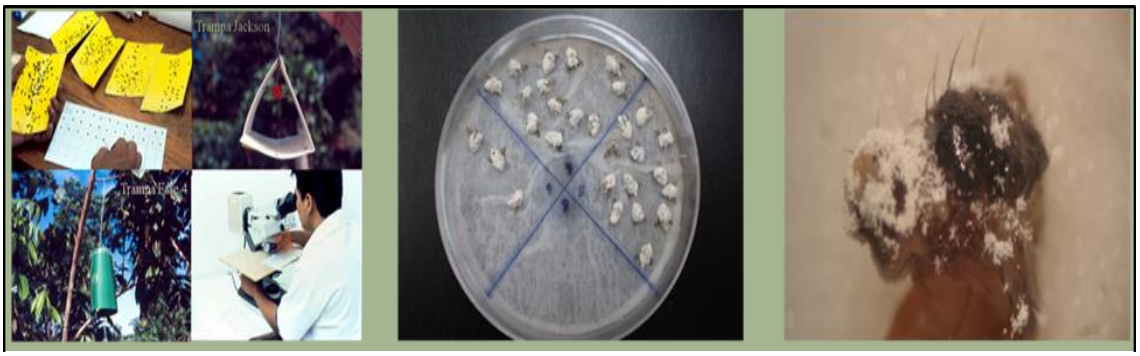
Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Use of Entomopathogenic Fungi for Fruit Fly Control in Area-Wide SIT Programmes



Food and Agriculture Organization of the United Nations

International Atomic Energy Agency

Vienna, 2019

DISCLAIMER

The proper citation for this document is:

FAO/IAEA. 2019. Use of Entomopathogenic Fungi for Fruit Fly Control in Area-Wide SIT Programmes, A. Villaseñor, S. Flores, S. E. Campos, J. Toledo, P. Montoya, P. Liedo and W. Enkerlin (eds.), Food and Agriculture Organization of the United Nations/International Atomic Energy Agency. Vienna, Austria. 43 pp.

Use of Entomopathogenic Fungi for Fruit Fly Control in Area-wide SIT Programmes

Antonio Villaseñor
IAEA/IPCS Consultant San Pedro La Laguna, Nayarit, México

Salvador Flores
Programa Moscafrut SADER-SENASICA, Metapa, Chiapas, México

Sergio E. Campos
Programa Moscafrut SADER-SENASICA, Metapa, Chiapas, México

Jorge Toledo
El Colegio de la Frontera Sur (ECOSUR), Tapachula, Chiapas, México

Pablo Montoya
Programa Moscafrut SADER-SENASICA, Metapa, Chiapas, México

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

Walther Enkerlin
Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria

Food and Agriculture Organization of the United Nations
International Atomic Energy Agency
Vienna, 2019

FOREWORD

Effective fruit fly control requires an integrated pest management approach which may include the use of area-wide sterile insect technique (SIT). For SIT to be cost-effective, the density of the wild population needs to be at low levels prior to the release of sterile insects. The required population level is achieved by the utilization of population suppression methods. This may include: insecticide-baits, bait stations, orchard sanitation practices and biocontrol agents including entomopathogens microorganisms. This manual is aimed at contributing the use of sterile flies as vectors of conidia of entomopathogenic fungi, for fruit fly suppression in operational programmes, as well as the so-called disseminator devices of conidia of entomopathogenic fungi.

This document presents a review of different studies that support the use of entomopathogenic fungi to suppress fruit fly populations. It describes the process to characterize pathogenic fungi strains, sterile fly the inoculation process, handling of sterile flies used as vectors, biosecurity recommendations and release densities of inoculated sterile flies, as well as the density of the disseminator devices. The integrated use of the SIT and Microbial Control (MC) as components of area-wide management strategies is presented. The FAO/IAEA Officer responsible for the publication was Walther R. Enkerlin.

CONTENT

1. INTRODUCTION

Entomopathogenic fungi have been reported as effective natural enemies of insect pest. In fruit flies there is empirical evidence that some common fungal species are efficient biological control agents. The purpose of this publication is to serve as a guide for the implementation of microbial control through the use of fruit flies as vectors of fungus conidia. Two methods are described: one is the direct inoculation of sterile fruit flies with spores and the other is the autoinoculation of wild fruit flies by conidia disseminator devices. In both methods, fruit flies become vectors or carriers of conidia that can be transfer to non-infected wild flies.

We present a review of different studies that support the use of entomopathogenic fungi to suppress fruit fly populations. Then we describe the process to characterize fungi strains. For inoculation process, the handle of flies and biosecurity recommendations are given to reduce the risk of contamination. Release densities of inoculated sterile flies or the density of disseminator devices are recommended for operational programs.

Finally, we propose the integrated use of the Sterile Insect Technique (SIT) and Microbial Control (MC) as components of area-wide management strategies.

2. BACKGROUND

Microorganisms that are insect pathogens represent an alternatives to be used as biocontrol agents (Mascarin & Jaronski 2016). In addition to the cost efficacy, the microorganisms are environmental, human health and non-target organism safe. Microsporidium fungi are primary pathogens that naturally affect insect pests, so they can be used in suppression of pest populations, mainly in conditions where the applications of chemical insecticides are ineffective or not feasible (Lacey & Kaya 2007). There is technology avaliable for the use of entomopathogens for the control of fruit flies of economic importance, within the Area-Wide Integrated Pest Management (AW-IPM) approach (Maniania et al. 2006, Maniania & Ekesi 2013, Flores et al. 2013, Toledo et al. 2017).

According to studies carried out in Algeria, Brazil, Canada, Colombia, Costa Rica, Spain, Egypt, Ghana, Greece, Guatemala, India, Indonesia, Mauritius, Italy, Morocco, Kenya, Mexico, Palestine, Pakistan, Thailand and South Africa, the fungus *Beauveria bassiana* Bals. (Vuill.) and *Metarhizium anisopliae* Mestch. (Sorokin), are two species of entomopathogens with greater potential for fruit fly control. The species of fruit flies involved have been *Anastrepha ludens* (Loew), *A. obliqua* (Mcquart), *A. fraterculus* (Wiedemann), *Bactrocera carambolae* Drew & Hancock, *B. dorsalis* (Hendel), *B. oleae* (Rossi), *B. zonata* (Saunders),

Ceratitis capitata (Wiedemann), *C. fasciventris* (Bezzi), *C. cosyra* (Walker), *C. rose* Karsch, *Rhagoletis cerasi* L., *R. indifferens* (Curran) and *Zeugodacus cucurbitae* Drew & Hancock. Between 2000 and 2018, at least 61 laboratory studies have been carried out, 8 in field cages and 13 in open field. Fifty studies were done with *B. bassiana*, 21 with *M. anisopliae* and 9 with isolates of *B. pseudobassiana* (Bals. Vuill.), *M. brunneum* (Petch), *M. quizhouense* (Chen & Guo) or *Isaria fumosorosea* (Wize) (See Appendix 1).

Current integrated pest management (IPM) of fruit flies includes the following measures: adult monitoring using traps, fruit sampling for monitoring of immature stages, chemical control with the application of insecticide baits, cultural and mechanical practices for sanitation, biological control based on parasitoid releases (e.g. *Diachasmimorpha longicaudata* (Ashmead)) and the release of sterile insects (Sterile Insect Technique, SIT) (Gutiérrez 2010).

The mass rearing of fruit flies in bio-factories for SIT application has been the basis of AW-IPM in countries such as the United States of America, Mexico, Guatemala, Argentina, Peru and others (Cáceres et al. 2000, 2014, Domínguez et al. 2010). The strategy for the proper functioning of the SIT is to continuously release large numbers of sterile insects, so that the ratio between released sterile flies and the wild flies is such that sterility is induced on the wild population leading to its reduction and eventually eradication (FAO/IAEA 2016). Nevertheless, in the case of high pest density is present in the field, in order for SIT to be cost-effective, the population of wild flies needs to be reduced often with the application of insecticide bait sprays (Liedo et al. 2010).

Application of insecticides such as Malathion for fruit fly control either aerial or by ground (Peck & McQuate 2000) can cause detrimental effects on the environment including mortality of non-target organisms (Leach & Mumford 2008). The use of more environmentally friendly compounds in bait sprays, such as spinosad, might represent an alternative to avoid or minimize these unwanted effects (USDA-APHIS 2000, Burns et al. 2001, Chueca et al. 2007).

In area-wide pest management programs where these environmentally friendly compounds are used, urban and suburban areas, ecological reserves, protected areas and lake basins are excluded (Vargas et al. 2008). In the Mediterranean Fruit Fly Control Program in Guatemala, coffee plantations associated with other crops, or when coffee is flowering are also excluded for bait sprays. All the unsprayed areas, represent potential reservoirs for the pest, from where fruit infestations will originate. Eventually, in these areas, pest

populations will reach high levels affecting the sterile to fertile ratio, and therefore, reducing the effectiveness of the SIT (FAO/IAEA 2016).

Microbial Control (MC) using entomopathogenic fungi is environmentally friendly, innocuous for humans and sustainable pest control measure (Zimmermann 2007a). When used in combination with fruit fly lures or sterile insects, it is highly specific. Therefore, MC is an ideal complement to other control tools (Butt et al. 2001).

Entomopathogenic fungi represent an effective pest control option in conventional and organic agricultural systems (Lomer et al. 2001, Meyling & Eilenberg 2007, Flores et al. 2013). The viability and transmission capacity of the pathogen determines the range of propagation in the host, according with the application method (Vega et al. 2000, Toledo et al. 2007, Quesada-Moraga et al. 2008). Studies on the viability and pathogenicity of the entomopathogenic fungi *B. bassiana* and *M. anisopliae* and how to apply them in the field have contributed to the development of MC technology for fruit flies (Maniania 1991).

The MC can be applied through both insect vectors and disseminator devices (Maniania 2002, Vega et al. 2000). As vectors, *C. capitata* sterile males inoculated with *B. bassiana* conidia where released over infested areas in Guatemala and their horizontal transmission to wild adults was demonstrated (Flores et al. 2013). Horizontal transmission was also demonstrated, when sterile inoculated flies were released in a medfly outbreak in the State of Chiapas, Mexico, adjacent to the Guatemala border (A. Villaseñor, personal communication 2014). On the other hand, conidia disseminator devices (autodisseminators) have been developed to attract and infect insect pests so that they later contaminate other conspecifics (Vega et al. 2000, Dimbi et al. 2003a). The dispersion of entomopathogenic conidia by means of disseminator devices and insect vectors have been proposed as alternatives for the control of fruit flies (Toledo et al. 2007, Toledo-Hernández et al. 2016), as well as in other dipteran of medical and veterinary importance, such as the tsetse fly (*Glossina* sp.) (Maniania & Ekesi 2013).

3. CHARACTERISTICS OF ENTOMOPATHOGEN FUNGI

Entomopathogenic fungi are microorganisms associated to insects that have been used as biological control agents. Their taxonomic classification is shown in Table 1.

The *Beauveria* species form simple and irregular conidiophores that end in vertices in the form of clusters, the conidiogenous cell with the globose base or bulging in a thinning in the upper part forms a curved zig-zag sterigma (Barnett & Hunter 1972), powdery appearance, and white cottony or creamy yellow color (Ferron 1981). *Beauveria bassiana* (Balsamo)

forms globose or subglobose conidia of 2-3 × 2.0- 2.5µ, with scarce conidiophores, rarely in compact clusters (Liu et al. 2001) (Figure 1).

Table 1. Taxonomic classification of entomopathogenic fungi

Kingdom:	Fungi
Division:	Ascomycota
Class:	Sordariomycetes
Order:	Hypocreales
Family:	Clavicipitaceae
Main Genera:	*Beauveria*
	Isaria
	Metarhizium
Important species	*B. bassiana* (Bals.) Vuillemin
	B. pseudobassiana S.A. Rehner & Humber
	I. fumosorosea Wize
	M. anisopliae (Metschn.) Sorokin
	M. brunneum (Petch)
	M. guizhouense Q.T. Chen & H.L. Guo

Beauveria bassiana is a natural inhabitant of the soil, an obligate parasite of several insect species (Zimmermann 2007b). The conidia or spores of the fungus penetrate normally in the cuticle of the insects, although they can enter the respiratory system via spiracles or via oral route when ingested. They feed on insect cells, their mycelial growth excreting toxins that kill the host insect. The stages that the fungi develop in their hosts are: germination, appressorium formation, formation of penetration structures, colonization and reproduction. The inoculum or infective unit is constituted by the structures of sexual and asexual reproduction, it means, spores and conidia. The infective process is described by Hajek & St. Lefger (1994), Sandhu et al. (2012), Valero-Jimenez et al. (2016). Moreover, the entomopathogenic fungus synthesize toxins that are used in the cycle of pathogen-host relationships. Among these toxins dextruxins, demetildextruxin and protodextruxin have been found, which are substances with toxic activity on insects, mites and nematodes (Monzon 2001).

Since entomopathogenic fungi are living organisms, for use in pest management programs, factors promoting the development of an epizootia must be understood to predict and manage the dynamics of the interaction of pest, pathogen and the environment. The pathogenicity of the strain against the target pest must be known, as well as the amount of spores required to induce an epizootia in the field. The effectiveness of pest control by

entomopathogens depends on the contact between the pest and the fungus, so the quality of the applied product is key (Wrigth et al. 2001).

The microbial control through the use of entomopathogenic fungi requires an established rearing that supplies the spores or conidia, in conditions that guarantee their viability (at least six months). Conidia are affected by light, humidity and high temperatures; so once harvested the fungus should be kept refrigerated to preserve its viability. During the production process, quality control tests guarantees yield, avoiding the loss of materials and reagents (Monzon 2001).

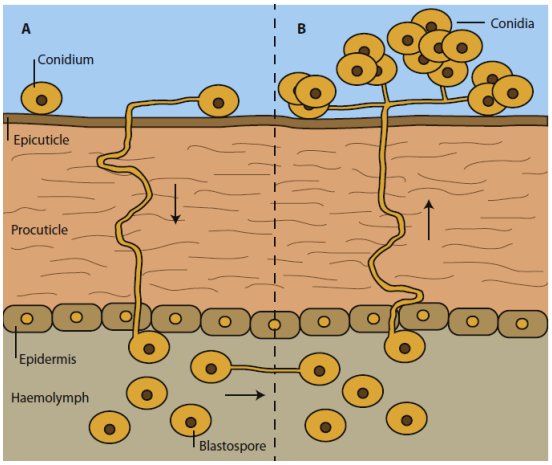


Figure 1. Transversal cut of insect cuticle with structural growing of *Beauveria bassiana* (Valero-Jimenez et al. (2016)

4. SELECTION OF PATHOGENIC STRAIN

For microbial control is recommended to use endemic strains collected in the same region, due to their adaptation to the environment. Searching in the field for infected insects (Orthoptera, Diptera, Coleoptera) has been the basis for collecting and establishing local stocks that can be maintained and multiplied at small scale under laboratory conditions (low production) or industrial laboratories (Monzon 2001).

Commercially available strains have standard production protocols (Toledo et al. 2017). Table 2 presents some commercial products based on entomopathogenic fungus from different parts of the world that can be evaluated to be used against fruit flies. This list is provided as a guidance. Do not represent specific recommendations, or that other products cannot be used. Any product should be tested for efficacy before applying it.

Once the fungus strain has been defined, the tests must be carried out with the target species of fruit flies which can be obtained from naturally infested fruits. If they are not sufficient for the evaluation, adults from a laboratory colony or mass rearing facility (fertile or sterilized fruit flies for SIT) can be used.

4.1. Viability tests

As a product coming from a living organisms, viability of entomopathogenic spores decreases when are stored for a long time. High temperatures are the most important factor affecting fungi viability, so they must be stored at 4 to 8 °C (Montesinos-Matías et al. 2015). They can be maintained at environmental conditions for short periods of time, in cool places, avoiding direct sun exposure.

For the conidia viability test, an aliquot of 1×10^6 to 1×10^8 conidia/ml must be prepared. The conidial concentration in the suspension is estimated by counting spores using a hemocytometer (Inglis et al. 2012). The agar immobilization method or Plate count method can be used to evaluate the germination of conidia. In the first method, the large area of a microscope slide is cover with potato-dextrose-agar culture medium (PDA) or Dextrose Saboraud Agar (DSA) and a drop of aliquot is placed in the center of the slide. In second method, the surface of PDA or SDA culture medium in a Petri dish is inoculated under sterile conditions with the aliquot. The slide or Petri dish with the samples are incubated at 25 ± 2 °C and after 15 h is reviewed through a microscope at 250×. Germination is defined as the point at which the germ tube length exceeded the spore diameter (Soylu et al. 2010). A strain is viable when it has more than 90% germinated conidia. The germination of conidia and the activity of the fungus are affected by factors such as ultraviolet radiation and environmental humidity. Conidia kept at room temperature may reduce its viability after 10 to 15 days, but formulated conidia (i.e. talcum powder, diatomite) remain viable at least 60 days. A correctly packed and sealed product protect the conidia from sunlight exposure and water penetration. In addition, diatomaceous earth or oils can increase the effectiveness of entomopathogens (Luz et al. 2012).

4.2. Pathogenicity and virulence test for fruit flies

The characterization and pathogenicity of fungus strains are evaluated through bioassays. For fruit flies, the immersion method is commonly used to inoculate the insects. The first step is to immobilize the insects by exposure to either cold temperatures (2 °C for 5 minutes) or application of CO_2 or another process that does not affect the fly survival.

Table 2. Commercial products based on entomopathogenic fungus.

Commercial Name	Fungus	Country
ATEC Beauveria	*Beauveria bassiana*	India
Balence	*Beauveria bassiana*	USA
Bioblast	*Metarhizium anisopliae*	USA
Bb Plus	*Beauveria bassiana*	South Africa
Betel	*Beauveria brongniartii*	France
Bibisav-2	*Beauveria bassiana*	Cuba
BioAct WG	*Isaria fumosoroseus*	Germany
Bio-Green Granules	*Metarhizium flavoviridae*	Australia
Bio-Cane Granules	*Metarhizium anisopliae*	Australia
Biogrubex	*Beauveria bassiana*	India
Biolarvex	*Beauveria bassiana*	India
Bioline	*Lecanicillium lecanii*	India
Bio-power	*Beauveria bassiana*	India
Biomagic	*Metarhiziun anisopliae*	India
Biosappex	*Lecanicillium lecanii*	India
Biovert Rich	*Lecanicillium lecanii*	India
Botanigard	*Beauveria bassiana*	USA
Boveril PL63	*Beauveria bassiana*	Brazil
Boverin	*Beauveria bassiana*	Ukraine
Engerlingspilz	*Beauveria brongniartii*	Switzerland
Green Muscle	*Metarhizium flavoviridae*	France
Green Guard	*Metarhizium anisopliae*	Australia
Met-52	*Metarhizium anisopliae*	Denmark
Myco-Jaal	*Beauveria bassiana*	India
Mycotrol	*Beauveria bassiana*	USA
Naturalis-L	*Beauveria bassiana*	USA
Ostrinil	*Beauveria bassiana*	France
PFR-97	*Isaria fumosoroseus*	USA
Preferal WG	*Isaria fumosorosea*	Belgium
Vertalec	*Lecanicillium longisporum*	Japan
Bea-Sin	*Beauveria bassiana*	Mexico
Pae-Sin	*Isaria fumosoroseus*	Mexico
Meta-Sin	*Metarhizium anisopliae*	Mexico
Beauvedieca	*Beauveria bassiana*	Costa Rica, Panama
Bazam	*Beauveria bassiana*	Honduras
Agronova	*Beauveria bassiana*	Colombia
Baubassil	*Beauveria bassiana*	Colombia
Beauveril	*Beauveria bassiana*	Colombia

Compiled from Mishra et al. (2015), Zimmermann (2007b, 2008), De Faria et al. (2007), Ramanujan et al. (2014), Kabalouk et al. (2010), Maina et al. (2018), Mascarin & Jaronski (2016).
This list is provided as a guidance. Do not represent specific recommendations, or that other products cannot be used. Any product should be tested for efficacy before applying it.

Groups from 20 to 50 flies are dipped in a test tube containing 1 ml of fungal suspension from 1×10^6 to 1×10^8 conidia / ml, and shaked gently for 30 seconds (Qazzaz et al. 2015). Subsequently, the flies are placed on absorbent paper into Petri dishes to remove excess moisture. The inoculated insects are sorted in groups of 20 flies in glass or Plexiglas cages of 30 × 30 × 30 cm with water and feed and maintained at 25 ± 2 °C and 70 - 80% relative humidity, and a photoperiod of 12 h light: 12 h darkness. For untreated control, the insects are submerged for 30 s in sterile distilled water. Dead flies are removed daily from cages, and they are immediately surface disinfected with a solution of 1% sodium hypochlorite for 5 s, followed by 3 rinses with sterile distilled water. Finally, the dead flies are transferred to a humidity chamber (glass Petri dish with a wet filter paper and sealed with Parafilm®) and kept in a room at 26 °C to promote the growth of the fungus on the dead bodies to corroborate that fungus infection was the cause of death (Wilson et al. 2017). The percentage of sporulation of the batch of 100 insects is calculated for each strain and for the control. This procedure is repeated at least with five different batches. Each batch can be considered as a replicate for the statistical analysis.

Virulence is represented by the Mean Lethal Time (LT_{50}) or by the Average Survival Time (AST). The percentage of sporulation is subjected to a Probit analysis (Finney 1952) from which the LT_{50} is estimated. A Log Rank analysis to sporulation data per day is applied to obtain the AST. The virulence of different batches of flies can be used as a replicate to compare among different fungus strains.

4.3. Determination of Mean Lethal Concentration (LC_{50})

The dose-mortality relationship is determined for the strain that showed the highest virulence against fruit flies. The concentrations to be evaluated can range from 10^4 to 10^8 conidia/ml plus a control. Flies are dipped for 30 s in fungus suspension following the procedure described above. Based on the initial fungus formulation, the concentration must be expressed in conidia/ml or conidia/fly. The number of conidia per fly is calculated dividing the total of conidia in the solution by the number of inoculated fruit flies. From each batch of flies, 100 insects are treated with each concentration and placed in five plastic containers of 2 liters (20 insects each). For 20 days, daily mortality is recorded. Dead insects are disinfected and placed in humid chambers to stimulate mycelium growth and confirm that death was caused by the fungus infection (as above in 4.2). For each concentration and for the control, the percent sporulation is calculated. The percentage is corrected by means of Abbott's formula (Abbott 1925), after which LC_{50} is estimated by Probit analysis. This procedure must be repeated with at least five batches of flies, and the lethal concentrations of each replicate can be used for statistical comparisons.

4.4. Characterization of strains

The virulence of each fungus strain is determined by the mean lethal time (LT_{50}) (De la Rosa et al. 2002) and it will depend on the insect from which the strain was isolated and the susceptibility of the insect where it was evaluated (Hajek & St. Leger 1994). The differences between strains or insect populations may be due to host-pathogen associations (Lecuona et al. 1996). Muñoz et al. (2009) evaluated 16 strains of *B. bassiana* against *C. capitata* reporting mortalities from 12.9 to 91.2% and lethal times from 3.83 to 17.64 days. De la Rosa et al. (2002) reported mortalities from 82 to 100% and lethal times from 2.82 to 5.99 days when evaluating seven strains of *B. bassiana* against *A. ludens*. Lezama-Gutiérrez et al. (2000) reported mortalities greater than 83.7% with *M. anisopliae* against *A. ludens*, and Hernández-Díaz-Ordaz et al. (2010) reported mortalities from 89.8 to 99.8 % evaluating two strains of *B. bassiana* and one of *M. anisopliae* against adults of *A. obliqua*. Osorio-Fajardo & Canal (2011) reported for two strains of *B. bassiana* and one of *M. anisopliae* lethal times of 42.7, 48.1, and 56 h, respectively, against *A. obliqua* and LC_{50} of 1.81×10^6 - 2.38×10^6 conidia/ml. Quesada-Moraga et al. (2006) reported LC_{50} values against *C. capitata* of 1×10^6 and 5.4×10^7 conidia/ ml with *B. bassiana*.

Toledo et al. (2007) evaluated two strains of *B. bassiana* on *A. ludens*: the LCPP (Local Committee on Plant Protection) strain at 1.0×10^8 conidia/ml produced a mortality of 98.7% with LT_{50} of 4.20 days and LC_{50} of 9.35×10^5. The Bassianil® strain product (Biotropic S.A. de C.V., Mexico) caused 99.3% mortality with LT_{50} of 4.04 days and LC_{50} of 2.69×10^7 conidia / ml. Against *C. capitata* values of LC_{50} of 6.86×10^7, 4.20×10^7 and 8.22×10^7 conidia/ml, and LT_{50} of 3.8, 4.10 and 4.2 days were reported for Bb-ET, GHA and Bb-AES strains of *B. bassiana,* respectively (Toledo et al. 2007). In Thailand, several strains of *B. bassiana* and *M. anisopliae* were evaluated against *B. dorsalis* adults, the results indicated that the most virulent strain produced 68% mortality and a LC_{50} of 7.36×10^7 conidia/ml (Aemprapa 2007).

4.5. Importance of LC_{50} and LT_{50}

The LC_{50} and LT_{50} are crucial parameters in the application of MC against fruit flies. The LC_{50} shows the approximate inoculum concentration so that insect vectors or disseminator devices can transmit the conidia during the interaction with other individuals. The LT_{50} indicates the median lethal time from pathogen exposure to death of an infected fly. Although flies gradually become sick, it is desirable that death occurs around four days after infection. During this time, fruit flies can disperse the inoculum that is attached to their bodies to other healthy flies through social interactions, for at least three days. Therefore, a *B. bassiana* strain of with an LT_{50} of four days will be recommended.

5. STERILE FRUIT FLIES AS VECTORS OF CONIDIA

The sexual behavior of fruit flies and the time required for inoculated fruit flies with entomopathogenic fungi such as *B. bassiana*, to reach the LT_{50}, make sterile flies good candidates for vectors of entomopathogens for use in biological control. Therefore, programs that apply the sterile insect technique (SIT) have a great potential to use MC. The developed technology for the release of chilled sterile flies, facilitates the inoculation process of the conidia of the entomopathogen, becoming the sterile flies on carriers of the fungus. In the case of the use of bait stations inoculated with conidia (disseminator devices), also non-inoculated released sterile flies will become vectors of the fungus when they get in contact with the bait station. The sexual behavior of fruit fly males, forming specific leks to attract females, facilitates the dissemination of the fungal spores among healthy and fertile individuals, resulting in a horizontal transmission and an exponential multiplicative effect of MC.

6. FRUIT FLY INOCULATION AND BIOLOGICAL SECURITY

In fruit fly mass rearing facilities, pupae are marked with fluorescent colored powder (Day-Glo Color, Cleveland, OH), sterilized using gamma-radiation and sent to the packing and release centers. Here the emerged sterile adults are held and fed until reaching sexual maturation and are ready for field release. Procedures are described in the Autocida Control Manual (Programa Moscamed 2015) and in the Guideline for Packing, Shipping and Release of Sterile Flies (FAO/IAEA 2017).

It is recommended that the pupae of flies to be used for the MC can be marked with a different color to be differentiated form flies used in regular SIT operations. The sterile flies are chilled at 2-5°C for 45 to 60 minutes depending on the volume, to immobilize them and collect them in plastic containers. In this step the sterile flies are inoculated.

6.1. Biosecurity procedures

The biosecurity procedures are divided in eight steps.

First. The staff involved in the inoculation activity must use safety equipment such as coveralls or gowns, latex gloves, face mask with dust filters.

Second. Prepare the doses of the entomopathogen before sterile pupae arrive to the packing and release facility. The doses should be prepared outside the facility. The spores will be placed in jars with screw caps. The recommended dose to inoculate *C. capitata* fruit flies is 8 g of powder (approx. 1.6×10^{10} conidia) per kilogram of sterile flies (≈ 150,000 flies)

(Flores et al. 2013, Toledo et al. 2017). This dose can be used as a baseline to determine the appropriate amount of formulate to be used for other fruit fly species, or for strain variations in the pathogenicity of the fungal strain or in its conidia concentration.

Third. The chilled flies are inoculated using an electric device equipped with an air compressor, a container for the conidia and a hose (Figure 2a and b). This device has a funnel to place the fruit flies in the PARC box. A gate at the bottom of funnel seals the PARC box during the application of conidia. The hose pass through the conidia container and carries the powder to a PARC box containing the sterile flies. The pump pressure is adjusted to 50 psi, and the required time to apply the required dose of conidia. In the case of the Mediterranean fruit fly, to apply each 8 g of conidia per Kg of flies, compressed air is injected during 16 s. Each PARC box must contain a maximum of 5 Kg of flies, so the time of air injection should be 80 s. The PARC box closed with the gate of the collector funnel, support the air injection with minimum release of conidia. This equipment allows inoculating the insects fast enough to avoid a significant increase of time in the chilling room, both for the staff personnel and for the inoculated sterile flies. The equipment is a prototype that must be improved to uniformly distribute the conidia and reduce the escape of conidia and personnel exposure.

2a

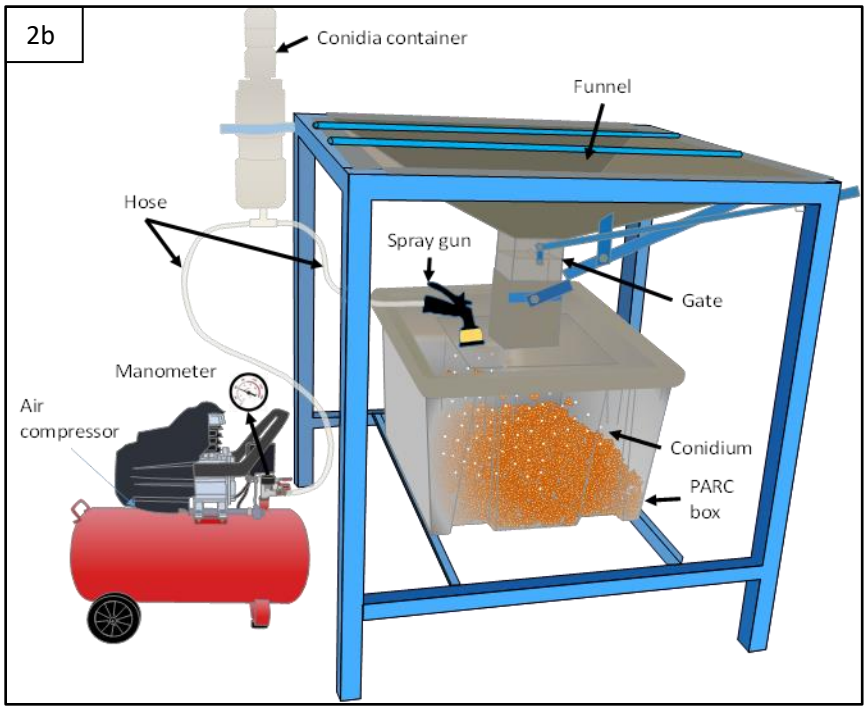


Figure 2a and b. Inoculation equipment used by the Moscamed Program, Mexico for the inoculation of *Ceratitis capitata* with conidia of *Beauveria bassiana*.

In other hand, for ground releases, the sterile flies are packed in PARC boxes or Kraft paper bags. In both cases, a hand-duster (mata formiga light duster, Guarany industries, Brazil) is used for inoculation. The tank is filled with formulated conidia, and a rubber hose is inserted into a hole in either a paper bag or a plastic PARC type box hermetically closed to prevent the leakage of conidia. Each pump stroke is equivalent to 0.5 g (i.e. 1×10^9 conidia for *B. bassiana* formulate) of powder (Figure 3).

Fourth. The bottles with the fungus doses, the boxes, any other containers and the equipment used for inoculation, must be clearly labeled "Exclusive use for inoculation process". All these equipment and materials should be stored and kept in separated rooms from those that will be used for sterile fly released aimed at applying the SIT.

Fifth. To minimize the risk of contamination, it is recommended that only one chilling room will be assigned for inoculation with entomopathognic fungi. The room must be clearly labeled in the outside "Inoculation room". When the sterile flies are being inoculated, the container with the flies (i.e. PARC box), should be rotated smoothly to allow a uniform distribution of the conidia.

Sixth. Before opening the PARC boxes (with the inoculated sterile flies), it is necessary to wait 5 minutes for dissipation of the powder. After that, inoculated flies are transferred to

a release box and mounted on the release machine in the plane. The release boxes with inoculated flies must be clearly labeled.

Seventh. - After inoculation, clothing and staff personal protective equipment, and all utensils, containers, equipment should be thoroughly washed with high pressured water, disinfected with a chlorine-based solution, then dried, ventilated and exposed to sunlight. The chilling room floor should be thoroughly washed with high pressure water and disinfected with chlorine. In addition, it is recommended the installation in the room of UV lamps which will remain on during four hours. This will allow free conidia in the working area to be inactivated

Eighth. Once the release of inoculated sterile flies is finished, the boxes and the releasing machines and the aircraft outlet tube must be washed with high pressured water, dried and exposed to sunlight for at least one hour to inactivate any remaining conidia.

7. DENSITY OF INOCULATED STERILE FLIES FOR AERIAL RELEASES

In some cases, empirical research on fruit flies has shown that sterile to fertile ratios equal to or greater than 60:1 are necessary to achieve suppression (Rendon et al. 2004, Shelly et al. 2005, Shelly & McInnis 2016). For this, the wild population should be at low density. Experience have demonstrated that under certain conditions when the flies per trap per day (FTD) is equal or below to 0.01, this value is obtained through the application of suppression methods such as aerial or terrestrial insecticide bait sprays, bait stations (mass trapping), biological control and mechanical control (fruit destruction) (FAO / IAEA 2017). The use of sterile flies inoculated with conidia of entomopathogenic fungi such as *B. bassiana* can be added to the list of effective methods to achieve population suppression.

The procedure for calibration of the release equipment to adjust the density of inoculated sterile flies is the same used for non-inoculated sterile flies. The initial release density of inoculated sterile flies will depend on the density of the wild population, expressed as FTD (FAO/IAEA 2017).

For Mediterranean fly, in suppression areas, an S: F ratio of 25-100: 1 is adequate. This ratio is will depend on the agroecological conditions in the area. Lower values (25: 1) can be employed in areas with low host density, and the higher values (100: 1) can be required in areas with high host density as in coffee growing. In areas where over-flooding ratio are not adequate for SIT, a population suppression method must be implemented prior releases (SENASICA-SAGARPA 2015, FAO / IAEA 2017). Then, MC can be used, since the inoculated sterile flies will be able to exert the suppression effect on the pest, until reaching the desired

wild FTD. In area-wide- programs with continuous host availability, the aerial release blocks must have a minimum of 1 000 ha to easily identify the flight lines. In smaller scale programs, or in hotspots, the release of inoculated sterile flies must be directed to the target area.

Considering that Shelly et al (2015) report 84% sterility at a 10 : 1 overflooding ratio, and at a 60 : 1 it increased to only 90% ,this overflooding range can be used for pest suppression. For eradication proposes, a higher overflooding must be required. We consider that for the release of Inoculated Sterile Flies (Si) and over-flooding ratio of at least 10:1 should be enough, since the aim of MC is to introduce the inoculum of entomopathogenic fungus in the wild population, so that through horizontal transmission and its multiplier effect, pest suppression can be achieved.

In the case of the Mediterranean fruit fly, since the LT_{50} of 4 days in the inoculated sterile flies, the release density must be adjusted each week until the FTD of the pest decreases at levels where SIT can be effective, in certain instances, an FTD level of 0.01 would be necessary. For suppression strategy, a continued period of sterile inoculated flies release for at least three pest generations (approximately twelve weeks) must be scheduled.

8. FRUIT FLY INOCULATION IN PAPER BAGS OR PARC BOXES

The pupae of sterile flies for MC and ground release must be marked with a fluorescent powder of a different color than used for aerial release in the SIT. For fly emergence, rectangular Kraft paper bags of No. 20 (9 × 21 × 12 cm) plus a paper filling support (100 × 20 cm) are used. Food is provided with a 30 × 20 cm paper strip impregnated with a mixture of 4% hydrolyzed protein and 96% sugar (6.270 cm^2 internal surface). Each bag should contain 12,540 sterile flies (2 flies / cm^2) for species with pupal sizes between 3-8 mg and 6,250 (1 flies / cm^2) for species with pupae of 9-12 mg. The bags are closed or tied with a rubber band to prevent flies scape. The bags are placed in the emergency room at 23 ± 1 °C for 4 days (FAO / IAEA 2017).

For holding in PARC boxes, pupae are distributed in 6 paper bags, and four 45 × 24 cm paper strip are placed, so that the total inner surface of the box is 29,000 cm^2 to contain either 2 flies/cm^2 for 3-5 mg pupae or 1 fly/cm^2 for 9-12 mg pupae (SENASICA-SAGARPA 2015). To feed the flies, the paper strips are impregnated with food prepared as described above. Ground releases are less recommended because distribution is not uniform (FAO/IAEA 2017).

On the day programed for field releases, flies are inoculated with fungus conidia. The inoculation is done with a manual-duster of one kilogram. The container in the lower part of the pump is filled with the amount conidia formulate to be applied in one day, considering 0.8 g per bag or 3.2 g per PARC box (i.e. 2×10^9 conidia per g for *B. bassiana* formulate). Before inoculation, the duster must be calibrated, to know the amount of formulate released per squeeze. When the pump piston is operated, the air pushes the powder through a rubber hose. The calibration is done by collect the pumped powder in a closed container, and the expelled conidia is weighted. To inoculate, the rubber hose is inserted through a hole in the bag or in the PARC box, through which the conidia is applied. The hole is covered with adhesive paper in the bags and a rubber stopper in the boxes, before and after the inoculation (Figure 3).

9. GROUND RELEASE

On the infested area, the release points are established using a GPS. The geo-referenced points will be recorded to facilitate the activity. The released density is designated by the number of flying flies that is obtained from each bag or box.

Figure 3. Medflies inoculation with a hand-duster “mata formiga” light duster (Guarany industries, Brazil) in PARC boxes and releasing of inoculated medflies.

The transport of the bags or boxes with inoculated flies should be done early in the morning in a vehicle with a camper to protect from the rain or the sun and start the release when there is already sunlight. Inoculated flies should not be exposed directly to sunlight to avoid affecting the viability of the fungus. The release of the flies must be done at each selected point by opening and tearing the bags or boxes so that the flies fly out. At low temperatures, the flies will take longer to fly out, it is recommended to remove the paper that was placed inside and shake it to release all the flies in the field. The empty puparia can be left in the

field, since these are organic matter that will be degraded. If preferred, can be taken to a place for organic waste.

10. QUALITY CONTROL

A bag of sterile flies from each batch that is released will be left in the packing center to determine the flight ability. This test will be done after 45 minutes of it being opened for the flies to leave. The percentage of absolute flyers will be calculated as a percentage of weight loss in the bag as described in the quality control manual (FAO/IAEA/USDA 2014).

To corroborate the pathogenicity of the fungus on the inoculated flies before the release, three 20-flies samples will be taken. These flies are placed in 30 × 30 × 30 cm Plexiglas cages with water and food. Daily mortality will be recorded for 20 consecutive days. The dead flies will be placed on absorbent paper, separated 2 cm among them and placed in Petri dishes to make a humid chamber. The dishes are maintained at 25 °C for 5 days (enough time for the fungi to be observed over the dead flies). In this way it is determined whether the cause of death is from the infection of the fungus or not. It is recommended to have a control of no-inoculated sterile flies from the same batch.

11. USE OF CONIDIA DISSEMINATORS

The use of bait stations to attract and infect fruit flies with an entomopathogenic fungus is an alternative method where sterile flies mass production infrastructure is no available or do not want to use sterile flies as carriers (Toledo et al. 2017).

The two main components of an infection station are: the device must be covered with an absorbent material for dust and conidia and contain a sexual or food attractant with high durability and low release rate. There could be male lures such as Trimedlure for species of the genus *Ceratitis* spp., methyl eugenol for *B. dorsalis* and related species, or Cuelure for *Z. cucurbitae* and related species. For *Anastrepha* spp. food attractants, such as hydrolyzed protein, enzymatic protein and synthetic attractants. The use of these devices is a strategy of "attraction and infection" given that the disseminator is baited with specific attractants, whether sexual, food or visual (Navarro-Llopis et al. 2015).

Currently, the Moscamed Regional Program has developed two bait stations to disseminate *B. bassiana* conidia for the control of wild populations of *C. capitata*. The cylindrical device is a 14.0 cm high × 8.5 cm in diameter, 500 ml plastic container (polyethylene terephthalate) with fifteen 2.5 mm holes evenly distributed on the wall, a lid containing four triangular openings 1.5 mm on each side and an open bottom. The canister with the TML plug is placed inside the device hanging from the top. The lid and the bottom are covered with tulle cloth

and the outer part of the device is covered with yellow plush (14 cm × 22 cm) where 2 g of conidia formulate (i.e. 2 × 10^9 per g conidia for *B. bassaina* formulate) of the fungus are applied (Figure 4a).

The second design is a 23 cm × 14 cm rectangular panel with a canister to place a 2 g TML plug inserted into a 2.5 hole in the center of the panel. It is also covered with yellow plush (23 cm × 14 cm) where 2 g of conidia formulate are placed (Figure 4b).

For homogenous dissemination of conidia in areas with *C. capitata*, one infection station per hectare should be installed and the plush with conidia should be replaced every 15 days (Flores et al. 2013).

The use of disseminator devices under the concept of "infection stations" is to suppress the pest or reduce pest population levels for effective performance of SIT. In a pilot project for Mediterranean fruit fly control, the sporulation values of wild flies were 57.3, 44.7 and 44.3% for treatments with sterile flies carriers, panel devices and cylindrical devices (one disseminator device per ha), respectively. The suppression of the wild population was on average 90% at the end of three months (Toledo et al. 2017).

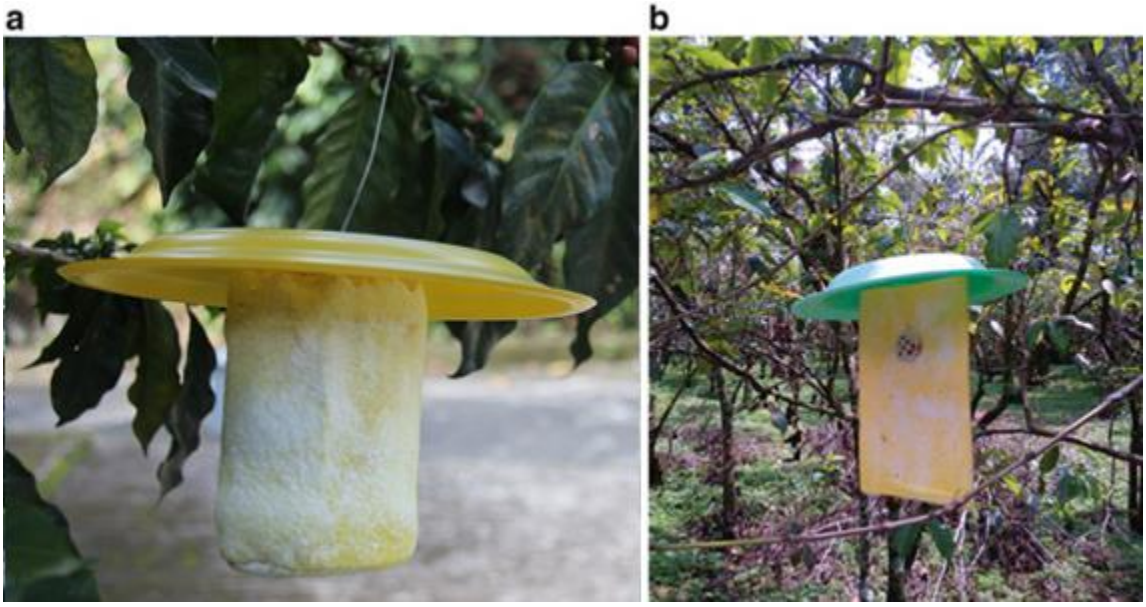


Figure 4. Cylindrical (a) rectangular panel (b) disseminator devices.

Disseminator devices have several advantages: 1) provide protection against ultraviolet rays to entomopathogenic fungi, increasing the viability of the conidia for a longer time; 2) increase the specificity to target pest when a specific bait is incorporated; 3) low cost, easy

to elaborate and maintain, 4) the amount of inoculum used in the devices is much less compared to the amount used for sprays for other pests (i.e. 2 g of *B. bassiana* formulate per ha with disseminators for Mediterranean fruit fly control, compared to 250 g of the formulate per ha for control of the coffee berry borer), and 5) infected insects propagate the pathogen during interactions with other individuals of the same species (Vega et al. 2000).

Using autodisseminator devices of *M. anisopliae* conidia, the population of the tsetse fly was reduced ~82%, indicating that the dispersion of the pathogen kept a low pest density (Maniania et al. 2006)

The use of autodisseminator devices is becoming more frequent due to its effectiveness. Klein & Lacey (1999) reported 95% of infection in *Popilia japonica* Newman by disseminator of *M. anisopliae* conidia. Maniania (2002) reported 100% infection of *M. anisopliae* in *Glossina fuscipes fuscipes* Newstead applied in a device made with a one liter capacity plastic canister fitted with a mosquito net with a wool cover to favor the contamination of the escape surface of the flies. The addition of a male lure or food attractant in the device, makes pathogen transmission more effective (Vega et al. 2007).

The strategy of dispersion of conidia of entomopathogenic fungi with disseminating devices is simple, economical and effective, so it has been proposed to apply it for the control of other species of fruit flies and the tsetse fly (*Glossinia* sp.) (Maniania & Ekesi 2013).

12. MONITORING OF FUNGUS TRANSMISSION

The traps and attractants used in surveillance of fruit flies are also used to monitor released sterile flies for SIT and released sterile flies for MC. In area-wide operational programs, the minimum density is one trap per 50 ha (2 traps/km^2) when a male lure is used and two traps per 50 ha (4 traps/km^2) when it is a food attractant. In orchards, at least 1 trap per 10 ha, up to 1 trap/ha can be used. The location of the traps should be georeferenced to facilitate the operation and management of information (FAO/IAEA 2018).

13. GROUND RELEASE AND SET UP OF DISSEMINATOR DEVICES AND TRAPS

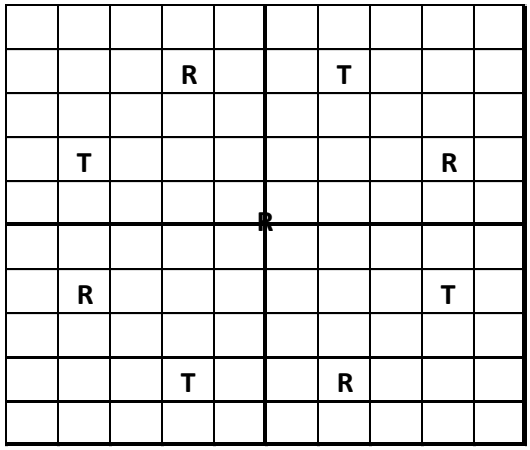
In fruit orchards, sterile fly ground release points should be evenly distributed among the traps, placing the release points at the approximate distance of natural fly dispersal. A good example could be to put five release points respect the four traps per square kilometer (Figure 4). In areas with an irregular host distribution, the number of traps and release points, as well as the distance between the release points, will be a function of the distribution and density of the hosts in the area of interest, always considering the dispersal

distance of the sterile flies released. This dispersal range, from 100 to 800 m is for different species of sterile fruit flies (Weldon et al. 2014).

In areas under population suppression, the main reservoirs of the pest with small to medium surfaces (1-50 ha), can be controlled using bait stations to attract and infect wild males to disseminate the entomopathogen conidia. In these small areas, at least one device must be installed. It is recommended to intersperse disseminators with the traps, following the model of Figure 5.

14. IMPACT OF MICROBIOLOGICAL CONTROL

Captures from inspected traps, are separated based on color marking and place in humid chambers (Petri dishes with absorbent paper saturated with sterile distilled water). The flies marked with the color for inoculated sterile flies, the flies marked with color for SIT, the flies not marked that are diagnosed as sterile and the unmarked wild flies. Petri dishes should be placed at 25 °C to promote the growth of the fungus for 5 days. On the sixth day the presence of the fungus is verified by means of observations using a stereoscope microscope. From these observation the percentage of transmission, based on the number of sporulated wild flies, is estimated (Figure 6).



T= Trap R= Release point

Figure 5. Distribution model of four traps and five ground release point for the microbial control of fruit flies (1 km^2). Each small square represents 1 ha (100 x 100 m).

The horizontal transmission of the entomopathogen to individuals of the wild population should be quantified in both sexes, although the capture of flies is influenced by the type of trap used during the monitoring. Therefore, it is recommended to install traps with food attractant and male lure.

The FTD values for sterile inoculated flies, with respect to FTD of infected and uninfected wild flies, will provide us the relationship between them, which will serve to make decisions on the operative strategies regarding the MC.

The percentages of distribution, measured as the number and percentage of traps with flies captured from each group (inoculated sterile flies, and infected and uninfected wild flies) will also provide us information about how the operational strategy is working on.

An example is shown in Table 3. It was a case of autoinoculation devices located on a coffee farm in Guatemala, which was under SIT application. The indexes of FTD indicated in general a good relationship sterile: fertile. In particular, also good relationship between sterile medflies and wild medflies, both infected through the autoinoculation devices. Also, the distribution is good in general and from regular to good was the distribution of the sterile medflies infected with respect to the distribution of the wild medflies. Although the distribution of infected wild medflies increases with time and the effect of MC application (Data base of Moscamed Program Guatemala 2016).

Figure 6. (left) Handle of captured flies on the traps, (center) humid chamber whit captured flies, and (right) adults of *C. capitata* with mycelial growth.

15. MICROBIOLOGICAL CONTROL AND INDUCED STERILITY

Although the target of MC using sterile flies as vectors is to suppress the pest by direct action of the fungus, it has the additional effect of introducing a certain degree of sterility that contributes to control of pest population. Roy et al. (2007), Dimbi et al. (2003a), Toledo et al. (2007) mentioned that the effectiveness of an IPM program can be increased if sterile flies are used as vectors of entomopathogenic organisms since, in addition to causing mortality to females through contamination during mating or mating attempts or through indirect horizontal transmission, they can introduce some degree of sterility in the wild population if the infected sterile males mate with wild females.

It has been demonstrated that infected sterile males of the Mexican fruit fly has a similar sexual performance during the first 3 days after the fungus inoculation than non-inoculated sterile males (Toledo et al. 2007). San Andrés et al. (2014) found that wild *C. capitata* females copulate in the same proportion with infected and uninfected males in leks, although they can remate when the first mate was with an infected male. The effectiveness of this strategy is enhanced when females remate from 4 to 28% in a wild population, because its increase the probability that females will mate with contaminated males that are active in the lek (Kraaijeveld et al. 2005). Dimbi et al. (2009) reported that the ability to copulate of males of three species of *Ceratitis* (*C. capitata, C. cosyra* and *C. fasciventris*) at

Table 3. Indices of evaluation by trapping, density and distribution of sterile medflies and its relationship with wild medflies both infected with *Beauveria bassiana* on autoinoculation devices at the San Isidro Chacaya coffee farm in Guatemala.

Week	FTD sterile	FTD fertile	Relationship S:F	FTD Sterile inoculated	FTD Fertile infected	Relationship S:F infected	FTD sterile uninfected	FTD fertile uninfected	Relationship S:F uninfected	Total Traps with capture (%)	Traps with sterile inoculated (%)	Traps with fertile infected (%)
7	0.3	0.0	11	0.3	0.0	9	0.1	0.0	0	60	20	0
8	0.6	0.1	7	0.2	0.1	4	0.4	0.0	14	80	80	0
9	0.9	0.1	11	0.9	0.1	16	0.1	0.0	2	80	80	0
10	2.3	0.1	40	1.5	0.0	53	0.8	0.0	27	80	0	60
11	1.7	0.4	4	1.2	0.1	11	0.5	0.3	2	80	80	60
12	4.0	0.3	13	2.4	0.2	11	1.6	0.1	19	80	20	80
13	1.0	0.1	9	0.5	0.1	6	0.5	0.0	18	80	60	60
14	3.9	0.0	137	2.7	0.0	96	1.2	0.0	0	40	40	40
15	8.3	0.0	0	7.0	0.0	0	1.3	0.0	0	60	20	60
16	3.6	0.1	63	0.7	0.0	0	2.9	0.1	51	100	60	100
17	1.7	0.0	61	1.7	0.0	59	0.1	0.0	0	60	10	60
18	0.4	0.0	0	0.3	0.0	0	0.2	0.0	0	100	60	100

0-2 days post-inoculation was not affected. In adults of *Z. cucurbitae*, the ability to copulate was not affected on the first day (Sookar et al. 2013). Moreover, fungal infection reduced both fecundity and fertility in wild females. Castillo et al. (2000) reported 65% of reduction in fertility of *C. capitata* treated with *I. fumosorosea* and 50% with *M. anisopliae*. Dimbi et al. (2003a) reported a reduction in fecundity but not in fertility in *C. capitata*, *C. cosyra* and *C. fasciventris* with *M. anisopliae*. Quesada-Moraga et al. (2006) indicate that the fecundity and the egg hatching in *C. capitata* were reduced when treated with *M. anisopliae*. When females of *A. ludens* were infected with *B. bassiana* through infected males, they recorded a significant reduction in fecundity, but fertility was not affected (Toledo et al. 2007, Sánchez-Roblero et al. 2012). However, Sookar et al. (2014a) reported that infection by *B. bassiana* in *B. zonata* and *Z. cucurbitae* did not affect the fertility.

When analyzing the sexual competitiveness of inoculated males, Sookar et al. (2014a) obtained a relative sterility index (RSI) of 0.59 with sterile males without inoculation and 0.44 for sterile males of *Z. cucurbitae* inoculated with *B. bassiana*. Novelo-Rincon et al. (2009) inoculated males of *A. ludens* 30 min before beginning the experiments and this did not significantly affect the sexual competitiveness of sterile flies.

In field cages, RSI of 0.40 and 0.42 were obtained for *B. bassiana* inoculated and non-inoculated Mediterranean fly sterile males, respectively. Horizontal transmission for both wild females and wild males was 72%. In Guatemala coffee plantations 3,000 inoculated sterile males per hectare per week were released by aircraft in a 7 km^2 (700 hectares). Throughout the study, the overflooding ratio was 26:1 sterile: fertile, and 44% of wild flies captured were infected with *B. bassiana* (Flores et al. 2013). This result indicated that inoculated sterile males interacted with wild flies in leks during courtship or copulation attempts. Therefore, it is assumed that the inoculated males can live the time required to perform a dual function, i.e., transmitting conidia between the wild flies and inducing sterility in the wild population (Novelo-Rincón et al. 2009). Higher transmission to wild males than to wild females can be due to, in lek, male-male interactions are more common than male-female interactions (Arita & Kaneshiro 1985). For suppression of *C. capitata* in coffee plantation by use of SIT, an overflooding ratio of 100: 1 at least is required (Rendon et al. 2004). However, a lower overflooding ratios (26:1 based on FTD values of sterile and wild flies) with releases of inoculated sterile males caused infection and death by fungal infection in 44% of the captured wild flies, which means that this strategy incorporates a new mortality component to the wild populations of *C. capitata*. The efficacy of entomopathogenic fungi to infect and kill adults of fruit flies is expressed in mortality, but also cause reductions on fecundity and fertility, which also contribute to suppression in the target population (Toledo et al. 2007, Sánchez-Roblero et al. 2012).

15.1. Knipling Model

Here we use the Knipling (1955) model to evaluate MC in the Mediterranean fruit fly. At 3: 1 and 10: 1sterile: fertile ratios, the impact of MC with sterile flies inoculated with conidia of *B. bassiana* is shown.

In field cage, a growth rate of 3.25 was obtained for the wild reference population. Four field-cage wild suppressed populations were: 1) Using sterile males at 3: 1 S: F ratio, the growth rate was 1.00. 2) At 3: 1 S: F ratio with inoculated sterile males, the growth rate decreased to 0.72. 3) At 10: 1 S: F ratio with sterile males, the growth rate was 0.16 and 4) at 10: 1 S: F ratio with inoculated sterile males, the growth rate was 0.02. It was considered that the reduction in the growth rate was an additional benefit for the transmission of the fungi (Toledo et al. 2014). Also, a field reference population with a growth rate of 5 was considered (Table 4).

An isolated Mediterranean fly population without immigration (field-cages) and exposed to an S: F of 3: 1 had a fertile couple index (FCI) of 0.60. While using sterile flies inoculated with *B. bassiana*, FCI was 0.62 (Flores 2015 unpublished data). Appling the Knipling model, with these parameters, sterile males reduced the population by 79% in the third generation (leaving 21% of the original population). And using inoculated sterile males, the population was reduced 92% (remaining only 8% of the original population). Under these conditions, the field-cage reference population would increase up to 2502% with an FCI of 0.90 and field reference population would increase 12 500% (Table 4).

15.2. Microbiological Control and Sterile Insect Technique Integration

In areas with high infestation levels or in hotspots, to use the SIT effectively, the first step is to suppress wild populations. The MC can be used for this purpose. The suppression of the population will occur mainly through the combined action of the sterile fly vector and the entomopathogen. Additional suppression can also be caused through the induction of sterility in the wild population by the inoculated sterile males. Once the appropriate wild FTD has been reached, the SIT must be applied through the release of sterile insects without inoculation for the purpose of maintaining pest populations at low levels to establish areas of low pest prevalence (ALPP) or for purposes of eradication to establish pest free areas (PFA). In the case of an ALPP, the SIT (release of sterile insects without inoculation) may continue as a component of management to maintain the phytosanitary status of the area.

Table 4. Population increase of *Ceratitis capitata* using the Knipling model considering the application of Sterile Insect Technique releasing *Beauveria bassiana* inoculated and no inoculated sterile males at S:F ratios of 3:1 and 10:1 in field cage evaluations.

Generation	Field reference population	field-cage reference population	Suppressed population in a ratio (S:F) 10:1	Suppressed population in a ratio (S:F) 10:1 using inoculated sterile males	Suppressed population in a ratio (S:F) 3:1	Suppressed population in a ratio (S:F) 3:1 using inoculated sterile males
Fertile couple index	1.0	0.9	0.60	0.62	0.60	0.62
REPRODUCTIVE RATE	5	2.93	0.10	0.01	1.00	0.45
P	300	300	225	225	300	300
F1	1,500	878	22	3	180	134
F2	7,500	2,567	2	0	108	60
F3	37,500	7,508	0	0	65	27
F4	187,500	21,960	0	0	39	12
F5	937,500	64,232	0	0	23	5
F6	4,687,500	187,878	0	0	14	2
F7	23,437,500	3,66,363	0	0	8	1
F8	117,187,500	1,071,610	0	0	5	0
F9	585,937,500	3,134,461	0	0	3	0
F10	2,929,687,500	9,168,298	0	0	2	0
F11	14,648,437,500	26,817,270	0	0	1	0

16. ACKNOWLEDGMENTS

The authors thank the Moscamed Mexico-Guatemala-United States Regional Program and the National Fruit Flies Program of Mexico, where large-scale sterile fly inoculation technologies were developed and applied and the MC's area-wide IPM management was demonstrated. We also thank the Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA), the Inter-American Institute of Cooperation for Agriculture (IICA), El Colegio de la Frontera Sur (ECOSUR), the Ministerio de Agricultura y Ganadería de Guatemala (MAGA), the United States Department of Agriculture (USDA) and the Food and Agriculture Organization (FAO) / International Atomic Energy Agency (IAEA) Joint Division. Their support allowed the development and pilot testing of the methods described here and their description for international public use. In the same way we acknowledge and thank all the scientists who from different countries who have made this technological innovation feasible and now is summarized in this work. Special acknowledgment to Rafael Argiles Herrero for revision of an earlier draft.

17. REFERENCES

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 3: 302–303.

Aemprapa, S. 2007. Entomopathogenic fungi screening against fruit fly in Thailand. Current Applied Science and Technology 7:122-126.

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

Amala, U., T. Jiji & A. Naseema. 2013. Laboratory evaluation of local isolate of entomopathogenic fungus, *Paecilomyces lilacinus* Thom Samson (ITCC 6064) against adults of melon fruit fly, *Bactrocera cucurbitae* Coquillett. Journal of Tropical Agriculture 51: 132-134.

Anagnou-Veroniki, M., D. C. Kontodimas, A. D. Adamopoulos, N. D. Tsimboukis & A. Voulgaropoulou. 2005. Effects of two fungal based biopesticides on *Bactrocera* (*Dacus*) *oleae* (Gmelin) (Diptera: Tephritidae). IOBC WPRS Bulletin 28: 49-51.

Arita L. H. & K. Y. Kaneshiro. 1985. The dynamics of the lek system and mating success in males of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.). Proceedings of Hawaiian Entomology Society. 25:39–48

Barnett H. L. & B. B. Hunter. 1972. Illustrated Genera of Imperfect Fungi. 3rd Ed. Burgess Publishing Co., Minneapolis, Minn. 241 p.

Beris, E. I., D. P. Papachristos, A. Fytrou, S. A. Antonatos & D. C. Kontodimas. 2013. Pathogenicity of three entomopathogenic fungi on pupae and adults of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). Journal of Pest Science 86: 275-284.

Boudjelida, H. & N. Soltani. 2011. Pathogenicity of *Metarhizium anisopliae* (Metsch) on *Ceratitis capitata* L. (Diptera: Tephritidae). Annals of Biological Research 2: 104-110.

Brito, B. D., A. L. Lima, K. R. Cruz, A. Bariani, C. R. Jesus-Barros, J. F. Pereira & R. Adaime. 2019. Amonian isolates of *Metarhizium* are effective for killing *Bactrocera carambolae* (Diptera: Tephritidae). Acta Biologica Colombiana 24:118-124

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

Butt, T. M., C. Jackson & N. Magan. 2001. Introduction – Fungal Biological Control Agents: Progress, Problems and Potential, pp: 1-8. In: T.M. Butt, C. Jackson & N. Magan (Eds.) Fungi as Biocontrol Agents: Progress, Problems and Potential. CAB International, London UK.

Cáceres, C., K. Fisher & P. Rendón. 2000. Mass rearing of the medfly temperature sensitive lethal genetic sexing strain in Guatemala, pp. 551–558. *In*: K. H. Tan (ed.), Proceedings: Area-Wide Control of Fruit Flies and Other Insect Pests. International Conference on Area-Wide Control of Insect Pests, and the 5th International Symposium on Fruit Flies of Economic Importance, 28 May–5 June 1998, Penang, Malaysia. Penerbit Universiti Sains Malaysia, Pulau Pinang, Malaysia.

Cáceres, C., J. Hendrichs & M. Vreysen. 2014. Development and improvement of rearing techniques for fruit flies (Diptera: Tephritidae) of economic importance. International Journal of Tropical Insect Science, 34(S1), S1-S12. doi:10.1017/S1742758414000034

Castillo, M. A., P. Moya, E. Hernandez & E. Primo-Yufera. 2000. Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts. Biocontrol 9: 274-282.

Chueca, P., H. Montón, J. L. Ripollés, P. Castañera, E. Moltó & A. Urbaneja. 2007. Spinosad bait treatments as alternative to malathion to control the Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae) in the Mediterranean Basin. Journal of Pesticides Science 32: 407–411.

Cossentine, J., H. Thistlewood, M. Goettel & S. Jaronski. 2010. Susceptibility of preimaginal western cherry fruit fly, *Rhagoletis indifferens* (Diptera: Tephritidae) to *Beauveria bassiana* (Balsamo) Vuillemin Clavicipitaceae (Hypocreales). Journal of Invertebrate Pathology 104: 105-109

Cossentine, J., S. Jaronski, H. Thistlewood & W. Yee. 2011. Impact of *Metarhizium brunneum* (Hypocreales: Clavicipitaceae) on pre-imaginal *Rhagoletis indifferens* (Diptera: Tephritidae) within and on the surface of orchard soil. Biocontrol Science and Technology 21: 1501-1505.

Daniel, C. & E. Wyss. 2008. Field applications of entomopathogenic fungi against *Rhagoletis cerasi*. *In*: Ecofruit-13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing: Proceedings to the Conference from 18thFebruary to 20th February 2008 at Weinsberg/Germany pp. 87-92.

Daniel, C. & E. Wyss. 2009. Susceptibility of different life stages of the European cherry fruit fly, *Rhagoletis cerasi*, to entomopathogenic fungi. Journal of Applied Entomology 133: 473-483.

De Faria, M.R. & Wraight S.P. 2007. Mycoinsecticides and mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. Biological Control, 43:237-256.

De la Rosa, W., F. L. López & P. Liedo. 2002. *Beauveria bassiana* as a pathogen of the Mexican fruit fly (Diptera: Tephritidae) under laboratory conditions. Journal of Economic Entomology 95: 36-43.

Destéfano, R. H. R., I. J. Bechara, C. L. Messias & A. E. Piedrabuena. 2005. Effectiveness of *Metarhizium anisopliae* against immature stages of *Anastrepha fraterculus* fruit fly (Diptera: Tephritidae). Brazilian Journal of Microbiology 36: 94-99.

Dimbi, S., N. K. Maniania, S. A. Lux & J. M. Mueke. 2003a. Host species, age and sex as factors affecting the susceptibility of the African tephritid fruit fly species, *Ceratitis capitata*, *C. cosyra* and *C. fasciventris* to infection by *Metarhizium anisopliae*. Journal of Pest Science 76: 113-117.

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

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

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

Dimbi, S., N. K. Maniania & S. Ekesi. 2013. Horizontal transmission of *Metarhizium anisopliae* in fruit flies and effect of fungal infection on egg laying and fertility. Insects 4: 206-216.

Domínguez, J., T. Artiaga-López, E. Solís & E. Hernández. 2010. Métodos de colonización de moscas de la fruta, pp. 259-276. *In*: P. Montoya, J. Toledo & E. Hernández (Eds.) Moscas de la Fruta: Fundamentos y Procedimientos para su Manejo. S y G Editores, México, D. F.

Ekesi, S., N. K. Maniania & S. A. Lux. 2002. Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. Biocontrol Science and Technology 12: 7-17.

Ekesi, S., N. K. Maniania & S. A. Lux. 2003. Effect of soil temperature and moisture on survival and infectivity of *Metarhizium anisopliae* to four tephritid fruit fly puparia. Journal of Invertebrate Pathology 83: 157-167.

Ekesi, S., N. K. Maniania, S. A. Mohamed & S. A. Lux. 2005. Effect of soil application of different formulations of *Metarhizium anisopliae* on African tephritid fruit flies and their associated endoparasitoids. Biological Control 35: 83-91.

Ekesi, S., M. De Meyer, S. A. Mohamed, M. Virgilio & C. Borgemeister. 2016. Taxonomy, ecology, and management of native and exotic fruit fly species in Africa. Annual Review of Entomology 61:219-238.

Elbashir, M. I., B. Paul, K. Shankarganesh, R. Gautami & P. Sharma. 2014. Pathogenicity of indian isolates of entomopathogenic fungus against important insect pests and natural enemies. Indian Journal of Entomology 76: 37-43.

FAO/IAEA. 2016. Guidelines for the use of mathematics in operational area-wide integrated pest management programmes using the sterile insect technique with a special focus on Tephritid fruit flies. Barclay H.L., Enkerlin W.R., Manoukis, N.C. Reyes-Flores, J. (eds.), Food and Agriculture Organization of the United Nations. Rome, Italy. 95 pp.

FAO/IAEA. 2017. Guideline for packing, shipping, holding and release of sterile flies in area-wide fruit fly control programmes. Rome, Italy, September 2017 [http://www-naweb.iaea.org/nafa/ipc/public/Guideline-for-Packing-Sept2017.pdf].

FAO/IAEA. 2018. Trapping guidelines for rea-wide fruit fly programmes. Rome, Italy, January 2018 [https://www.iaea.org/sites/default/files/trapping-guideline.pdf].

FAO/IAEA/USDA. 2014. Manual for Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies, Version 5.0. International Atomic Energy Agency, Vienna, Austria. 85 pp.

Ferron, P. 1981. Pest control by the fungi *Beauveria* and *Metarhizium,* pp. 45-55. *In*: H. D. Burges (Ed.) Microbial Control of Pest and Plant Diseases 1978-1980. Academic Press. USA.

Finney, D. J. 1952. Probit Analysis. Cambridge, England, Cambridge University Press.

Flores, S., S. Campos, A. Villaseñor, A. Valle, W. Enkerlin, J. Toledo, P. Liedo & P. Montoya. 2013. Sterile males of *Ceratitis capitata* (Diptera: Tephritidae) as disseminators of *Beauveria bassiana* conidia for IPM strategies. Biocontrol Science and Technology 23: 1186-1198.

Gandarilla-Pacheco, F. L., H. D. Nava-González, K. Arévalo-Niño, L. J. Galan-Wong, M. Elías-Santos & I. Quintero-Zapata. 2012. Evaluation of native strains of *Isaria fumosorosea* (Wize) against *Anastrepha ludens* (Loew) (Diptera: Tephritidae). Journal of Life Sciences 6: 957-960.

Goble, T. A., J. F. Dames, M. P. Hill & S. D. Moore. 2011. Investigation of native isolates of entomopathogenic fungi for the biological control of three citrus pests. Biocontrol Science and Technology 21: 1193-1211.

Gul, H. T., S. Freed, M. Akmal & M. N. Malik. 2015. Vulnerability of different life stages of *Bactrocera zonata* (Tephritidae: Diptera) against entomogenous fungi. Pakistan Journal of Zoology 47: 307-317.

Gutiérrez, J. M. 2010. El Programa Moscas de la Fruta en Mexico, pp. 3-10. *In*: P. Montoya, J. Toledo & E. Hernández (Eds.) Moscas de la Fruta: Fundamentos y Procedimientos para su Manejo. S y G Editores, México, D. F.

Hadi, M. S., T. Himawan & L. Q. Aini. 2013. The effectiveness of entomopathogenic fungi *Beauveria bassiana* with the addition of insect growth regulator Lufenuron for controlling *Bactrocera carambolae*. Journal of Tropical Life Science 3: 187-192.

Hadi, M. S., T. Himawan & L. Q. Aini. 2017. The application of *Beauveria bassiana* and Lufenuron could reduce the reproduction of fruit fly (*Bactrocera carambolae*) (Drew & Hancock) (Diptera: Tephritidae). Journal of Tropical Plant Protection 1:22-29.

Hajek, A. E. & R. J. St Leger. 1994. Interactions between fungal pathogens and insect hosts. Annual Review of Entomology 39: 293-322.

Hallouti, A., A. Zahidi, R. Bouharroud, A. El Mousadik, A. A. B. Aoumar & H. Boubaker. 2017. Diversity of entomopathogenic fungi in argane forest soil and their potential to manage Mediterranean fruit fly (*Ceratitis capitata*). Journal of Pharmacy and Pharmacology 5: 746-754.

Hernández Díaz-Ordaz, N., N. Pérez & J. Toledo. 2010. Patogenicidad de tres cepas de hongos entomopatógenos a adultos de *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) en condiciones de laboratorio. Acta Zoologica Mexicana 26: 481-494.

Ibrahim, A. A., N. A. Soliman, M. M. El-Deen, N. F. Ramadan & S. R. Farag. 2014. Susceptibility of the peach fruit fly, *Bactrocera zonata* (Saunders) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) adults to the entomopathogenic fungi; *Metarhizium anisopliae* (Met.) and *Beauveria bassiana* (Bals.). Egyptian Journal of Biological Pest Control 24:491-495.

Inglis, G. D., J. Enkerli & M. S. Goettel. 2012. Laboratory techniques used for entomopathogenic fungi: Hypocreales, pp. 189-253. *In* L. A. Lacey (Ed.) Manual of Techniques in Invertebrate Pathology. Second Edition. Academic Press. USA.

Imoulan, A. & A. Elmeziane. 2014. Pathogenicity of *Beauveria bassiana* isolated from Moroccan Argan forests soil against larvae of *Ceratitis capitata* (Diptera: Tephritidae) in laboratory conditions. World Journal of Microbiology and Biotechnology 30: 959-965.

Imoulan, A., S. K. Ibnsouda & A. El Meziane. 2016. Molecular characterization and the effectiveness of native entomopathogenic *Beauveria bassiana* strains against adults of Mediterranean fruit fly (*Ceratitis capitata*). Journal of Biosciences & Biotechnological Discovery 1: 6-16.

Jiji, T., R. Praveena, K. Babu, A. Naseema & N. Anitha. 2006. Three promising fungal strains pathogenic to fruit flies, pp. 175-177. In: Fruit Flies of Economic Importance: From Basic to Applied Knowledge Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance 10-15 September 2006, Salvador, Brazil.

Kabaluk, J. T., A. M. Svircev, M. S. Goettel & S. G. Woo (eds.) 2010. The use and regulation of microbial pesticides in representative jurisdictions worldwide. International Organization for Biological Control of Noxious Animals and Plants (IOBC) 99pp.

Klein M. G. & L. A. Lacey. 1999. An attractant trap for the autodissemination of entomopathogenic fungi into populations of the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). Biocontrol Science and Technology 9: 151-158.

Knipling, E. F. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. Journal of Economic Entomology 48: 459–462.

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

Kraaijeveld, K., B. I. Katsoyannos, M. Stavrinides, N. A. Kouloussis & T. Chapman. 2005. Remating in wild females of the Mediterranean fruit fly, *Ceratitis capitata*. Animal Behaviour 69: 771–776.

Lacey, L. A. & H. K. Kaya 2007. Field Manual of Techniques in Invertebrate Pathology: Application and evaluation of pathogens for control of insects and other invertebrate pests. Springer. Netherland

Ladurner, E., M. Benuzzi, F. Fiorentini & S. Franceschini. 2008. *Beauveria bassiana* strain ATCC 74040 (Naturalis®), a valuable tool for the control of the cherry fruit fly (*Rhagoletis cerasi*), pp. 93-97. In: Ecofruit-13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing: Proceedings to the Conference from 18thFebruary to 20th February 2008 at Weinsberg/Germany.

Leach A.W. & J.D. Mumford. 2008. Pesticide environmental accounting: a method for assessing the external costs of individual pesticide applications. Environmental Pollution 151: 139-147.

Lecuona, R. 1996. Microorganismos patógenos empleados en el control microbiano de insectos plaga. IMYZA–CICA–INTA Castelar, Buenos Aires. 338 p.

Lezama-Gutiérrez, R., A. Trujillo-de la Luz, J. Molina-Ochoa, O. Rebolledo-Domínguez, A. R. Pescador, M. Lopez-Edwards & M. Aluja. 2000. Virulence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) on *Anastrepha ludens* (Diptera: Tephritidae): laboratory and field trials. Journal of Economic Entomology. 93: 1080–1084.

Liedo, P., W. Enkerlin & J. Hendrichs. 2010. Fundamentos de la Técnica del Insecto Estéril, pp. 243-256. In: P. Montoya, J. Toledo & E. Hernández (Eds.) Moscas de la Fruta: Fundamentos y Procedimientos para su Manejo. S y G Editores, México, D. F.

Liu, Z. Y., Z. Q. LiangI, A. J. S. Whalley, A. Y. Liul & Y. J. Yao. 2001. A new species of Beauveria, the anamorph of *Cordyceps sobolifera*. Fungal Diversity 7: 63–72.

Lomer, C. J., R. P. Bateman, D. L. Johnson. J. Langewald & M. Thomas. 2001. Biological control of locusts and grasshoppers. Annual Review of Entomology 46: 667–702.

Lozano-Tovar, M. D., I. Garrido-Jurado, F. Lafont & E. Quesada-Moraga. 2015. Insecticidal activity of a destruxin-containing extract of *Metarhizium brunneum* against *Ceratitis capitata* (Diptera: Tephritidae). Journal of Economic Entomology 108: 462-472.

Luz, C., J. Rodrigues, and L. F. N. Rocha. 2012. Diatomaceous earth and oil enhance effectiveness of *Metarhizium anisopliae* against *Triatoma infestans*. Acta Tropica 122: 29-35.

Mahmoud, M. F. 2009a. Pathogenicity of three commercial products of entomopathogenic fungi, *Beauveria bassiana*, *Metarhizum anisopilae* and *Lecanicillium lecanii* against adults of olive fly, *Bactrocera oleae* (Gmelin)(Diptera: Tephritidae) in the laboratory. Plant Protection Science 45: 98-102.

Mahmoud, M. F. 2009b. Susceptibility of the peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) to three entomopathogenic fungi. Egyptian Journal of Biological Pest Control 19: 169-175.

Maina, U. M., I. B. Galadima, F. M. Gamba & D. Zakaria. 2018. A review on the use of entomopathogenic fungus in the management of insect pest of field crops. Journal of Entomology and Zoology Studies 6:27-32.

Maniania, N. K. 1991. Potential of some fungal pathogens for the control of pests in the tropics. International Journal of Tropical Insect Science 12: 63–76-

Maniania N. K. 2002. A low-cost contamination device for infecting adult tsetse, *Glossina* spp., with the entomopathogenic fungus *Metarhizium anisopliae* in the field. Biocontrol Science and Technology 12: 59-66.

Maniania N. K. & S. Ekesi. 2013. The use of entomopathogenic fungi in the control of tsetse flies. Journal of Invertebrate Pathology. 112: 583-588.

Maniania, N. K., S. Ekesi, A. Odulaja, M. A. Okech & D. J. Nadel. 2006: Prospects of a fungus-contamination device for the control of tsetse fly *Glossina fuscipes fuscipes*. Biocontrol Science and Technology 16: 129-139

Mar, T. T. & S. Lumyong. 2012. Evaluation of effective entomopathogenic fungi to fruit fly pupa, *Bactrocera* spp. and their antimicrobial activity. Chiang Mai Journal of Science 39: 464-477.

Marri, D., D. A. Gomez, D. D. Wilson, S. Y. Billah & M. Osae. 2016. Evaluation of the efficacy of a commercial formulation of *Beauveria bassiana* for the control of the invasive fruit fly *Bactrocera dorsalis* (Diptera: Tephritidae). Biopesticides International 12: 9-18.

Mascarin, G. M. & S. T. Jaronski. 2016. The production and uses of *Beauveria bassiana* as a microbial insecticide. World Journal of Microbiology and Biotechnology 32: 177

Medina, P., E. Corrales, M. González-Nuñez, G. Smagghe & E. Viñuela. 2008. Effects of *Beauveria bassiana*, *Heterorhabditis bacteriophora*, *H. megidis* and *Steinernema feltiae* on the Mediterranean fruit fly *Ceratitis capitata* and the very sensitive braconid Psyttalia concolor in the lab. IOBC/wprs Bulletin 35: 113-121.

Meyling, N. V. & J. Eilenberg. 2007. Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control. Biological Control 43: 145-155

Mishra, J., S. Tewari, S. Singh & N. K. Arora. 2015. Biopesticides: where we stand? pp. 37-75. *In* Arora N. K. (ed.) Plant microbes symbiosis: applied facets. Springer, New Delhi.

Mochi, D. A., A. C. Monteiro, S. A. De Bortoli, H. O. Dória & J. C. Barbosa. 2006. Pathogenicity of *Metarhizium anisopliae* for *Ceratitis capitata* (Wied.)(Diptera: Tephritidae) in soil with different pesticides. Neotropical Entomology 35: 382-389.

Montesinos-Matías, R., M. A. Ayala-Zermeño & A. M. Berlanga-Padilla. 2015. Manual para la conservación y mantenimiento de hongos entomopátogenos. Centro Nacional de Referencia de Control Biológico. Dirección General de Sanidad Vegetal. SAGARPA-SENASICA. Tecomán, Colima, México. pp. 59.

Monzón, A. 2001. Producción, uso y control de calidad de hongos entomopatógenos en Nicaragua. Manejo Integrado de Plagas (Costa Rica) 63: 95 - 103.

Muñiz-Reyes, E., A. W. Guzmán-Franco, J. Sánchez-Escudero & R. Nieto-Angel. 2014. Occurrence of entomopathogenic fungi in tejocote (*Crataegus mexicana*) orchard soils and their pathogenicity against *Rhagoletis pomonella*. Journal of Applied Microbiology 117: 1450-1462.

Muñoz, J. A., W. de la Rosa & J. Toledo. 2009. Mortalidad en *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) por diversas cepas de *Beauveria bassiana* (Bals.) Vuillemin, en condiciones de Laboratorio. Acta Zoológica Mexicana 25: 609-624.

Navarro-Llopis, V., I. Ayala, J. Sanchis, J. Primo & M. D. P. Moya. 2015. Field efficacy of a *Metarhizium anisopliae*-based attractant contaminant device to control *Ceratitis capitata* (Diptera: Tephritidae). Journal of Economic Entomology 108: 1570-1578.

Ndii, A. M., B. T. Rahardjo & T. Himawan. 2016. The combination of entomopathogenic fungus of *Beauveria bassiana* (Balls) Vuill. with the insect growth regulator (IGR) of Lufenuron against reproductive of *Bactrocera carambolae* fruit flies (Diptera: Tephritidae). The Journal of Experimental Life Science 6: 25-28.

Novelo-Rincón, L. F., P. Montoya, V. Hernández-Ortíz, P. Liedo & J. Toledo. 2009. Mating performance of sterile Mexican fruit fly *Anastrepha ludens* (Diptera: Tephritidae) males used as vectors of *Beauveria bassiana* (Bals.) Vuill. Journal of Applied Entomology 133: 702-710.

Oreste, M., N. Baser, G. Bubici & E. Tarasco. 2015. Effect of *Beauveria bassiana* strains on the *Ceratitis capitata-Psyttalia concolor* system. Bulletin of Insectology 68: 265-272.

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

Osorio-Fajardo, A. & N. A. Canal. 2011. Selección de cepas de hongos entomopatógenos para el manejo de *Anastrepha obliqua* (Macquart, 1835) (Diptera: Tephritidae) en Colombia. Revista de la Facultad Nacional de Agricultura de Medellín 64: 6129-6139.

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

Porras, L. & R. Lecuona. 2008. Estudios de laboratorio para el control de *Ceratitis capitata* (Wiedmann) (Diptera: Tephritidae) (Mosca del Mediterráneo) con *Beauveria bassiana*. Agronomía Costarricense 32: 119-128.

Programa Moscamed. 2015. Manual de procedimientos para el control autocida de la Mosca del Mediterráneo, *Ceratitis capitata* (Wiedemann). SAGARPA-MAGA-USDA. 63 pp.

Qazzaz, F. O., M. I. Al-Masri & R. M. Barakat. 2015. Effectiveness of *Beauveria bassiana* native isolates in the biological control of the Mediterranean fruit fly (*Ceratitis capitata*). Advances in Entomology 3: 44-55.

Queiroz de Oliveira, F., J. de Luna Batista, J. Bruno Malaquias, D. A. Almeida & R. de Oliveira. 2010. Determinación de la concentración letal media (CL_{50}) de micoinsecticidas para el control de *Ceratitis capitata* (Diptera: Tephritidae). Revista Colombiana de Entomología 36: 213-217.

Quesada-Moraga E., A. Ruiz-Garcia & C. Santiago-Alvarez. 2006. Laboratory evaluations of entomopathogenic fungi *Beauveria bassiana* and *Metarhiziuum anisopliae* against puparia and adults of *Ceratitis capitata* (Diptera: Tephritidae). Journal of Economic Entomology 99: 1955-1966.

Quesada-Moraga, E., I. Martin-Carballo, I. Garrido-Jurado. & C. Santiago-Alvarez. 2008. Horizontal transmission of *Metarhizium anisopliae* among laboratory populations of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Biological Control 47: 115-124.

Rashad, M. M., A. H. El-Heneidy, K. Djelouah, N. Hassan & S. A. Shaira. 2015. On the pathogenicity of entomopathogens to the peach fruit fly, *Bacterocera zonata* (Saunders) (Diptera: Tephritidae). Egyptian Journal of Biological Pest Control 25:649-654.

Rendón P., D. McInnis, D. Lance & J. Stewart 2004. Medfly (Diptera: Tephritidae) genetic sexing: large-scale field comparison of males-only and bisexual sterile fly releases in Guatemala. Journal of Economic Entomology 97: 1547-1553.

Roy H. E., J. Baverstock & J. K. Pell. 2007. Manipulating behaviour: a strategy for pest control? pp. 179–195 *In*: S. Ekesi & N. K. Maniania (eds.) Use of Entomopathogenic Fungi in Biological Pest Management. Research, SignPost, Kerala, India.

San Andrés V., I. Ayala, M. C. Abad, J. Primo, P. Castañera & P. Moya. 2014. Laboratory evaluation of the compatibility of a new attractant contaminant device containing *Metarhizium anisopliae* with *Ceratitis capitata* sterile males. Biological Control 72: 54–61.

Sánchez-Roblero, D., G. Huerta-Palacios, J. Valle, J. Gómez & J. Toledo. 2012. Effect of *Beauveria bassiana* on the ovarian development and reproductive potential of *Anastrepha ludens* (Diptera: Tephritidae). Biocontrol Science and Technology 22: 1075-1091.

Sandhu, S. S., A. K. Sharma, V. Beniwal, G. Goel, P. Batra, A. Kumar, S. Jaglan, A. K. Sharma & S. Malhotra. 2012. Myco-Biocontrol of Insect Pests: Factors Involved, Mechanism, and Regulation. Journal of Pathogens 2012: DOI 126819

SENASICA-SAGARPA 2015. Manual técnico para las operaciones de campo de la Campaña Nacional contra Moscas de la Fruta. Seccion V: Control Autocida. México. 26 pp. https://www.gob.mx/cms/uploads/attachment/file/262619/MT_Operaciones_de_campo_CNMF_Secci_n_V_CA.pdf

Shelly, T. & D. McInnis. 2016. Sterile insect technique and control of tephritid fruit flies: do species with complex courtship require higher overflooding ratios? Annals of the Entomological Society of America 109: 1–11.

Shelly, T. E., D. O. McInnis & P. Rendon. 2005. The sterile insect technique and the Mediterranean fruit fly: assessing the utility of aromatherapy in large field enclosures. Entomologia Experimentalis et Applicata 116: : 199–208.

Soliman, N. A., A. A. Ibrahim, M. M. El-Deen, N. F. Ramadan & S. R. Farag. 2014. Entomopathogenic nematodes and fungi as biocontrol agents for the peach fruit fly, *Bactrocera zonata* (Saunders) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) soil borne-stages. Egyptian Journal of Biological Pest Control 24:497-502.

Sookar, P., S. Bhagwant & E. Awuor-Ouna. 2008. Isolation of entomopathogenic fungi from the soil and their pathogenicity to two fruit fly species (Diptera: Tephritidae). Journal of Applied Entomology 132: 778-788.

Sookar, P., S. Bhagwant & M. N. Allymamod. 2010. Mortality in tephritid fruit fly puparia and adults caused by *Metarhizium anisopliae*, *Paecilomyces fumosoroseus* and *Beauveria bassiana*. University of Mauritius Research Journal 16: 281-298.

Sookar P., S. Bhagwant, F. B. Khayratee, Y. Choonea & S. Ekesi. 2013. Mating compatibility of wild and sterile melon flies, *Bactrocera cucurbitae* (Diptera: Tephritidae) treated with entomopatogenic fungi. Journal of Applied Entomology 138:409-417.

Sookar, P., M. Alleck, N. Ahseek & S. Bhagwant. 2014a. Sterile male peach fruit flies, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), as a potential vector of the entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin in a SIT programme. African Entomology 22: 488-498.

Sookar, P., S. Bhagwant & M. N. Allymamod. 2014b. Effect of *Metarhizium anisopliae* on the fertility and fecundity of two species of fruit flies and horizontal transmission of mycotic infection. Journal of Insect Science 14: 1-12.

Soylu, E.M., K. Şener & S. Soner. 2010. In vitro and in vivo antifungal activities of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*. International Journal of Food Microbiology 143: 183–189.

Thaochan, N. & A. Ngampongsai. 2015. Effects of autodisseminated *Metarhizium guizhouense* PSUM02 on mating propensity and mating competitiveness of *Bactrocera cucurbitae* (Diptera: Tephritidae). Biocontrol Science and Technology 25: 629-644.

Toledo, J., S. E. Campos, S. Flores, P. Liedo, J. F. Barrera, A. Villaseñor & P. Montoya. 2007. Horizontal transmission of *Beauveria bassiana* in *Anastrepha ludens* (Diptera: Tephritidae) under laboratory and field cage conditions. Journal of Economic Entomology 100: 291-297.

Toledo, J., P. Liedo, S. Flores, P. Montoya, S. E. Campos & A. Villaseñor. 2008. Use of *Beauveria bassiana* and *Metarhizium anisopliae* for fruit fly control: a novel approach, pp. 127-132. *In*: Fruit Flies of Economic Importance: From Basic to Applied Knowledge Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance 10-15 September 2006, Salvador, Brazil.

Toledo. J., S. Flores, S. Campos, A. Villaseñor, W. Enkerlin, A. Valle, P. Montoya & P. Liedo. 2014. Vertical and horizontal transmission of *Beauveria bassiana* under field conditions for *Ceratitis capitata* control, pp. 215. *In*: 9th International Symposium on Fruit Flies of Economic Importance (ISFFEI), Bangkok, Thailand, 12-16 May 2014.

Toledo, J., S. Flores, S. Campos, A. Villaseñor, W. Enkerlin, P. Liedo, A. Valle & P. Montoya. 2017. Pathogenicity of three formulations of *Beauveria bassiana* and efficacy of autoinoculation devices and sterile fruit fly males for dissemination of conidia for the control of *Ceratitis capitata*. Entomologia Experimentalis et Applicata 164: 340-349.

Toledo-Hernández, R. A., J. Ruíz-Toledo, J. Toledo & D. Sánchez. 2016. Effect of three entomopathogenic fungi on three species of stingless bees (Hymenoptera: Apidae) under laboratory conditions. Journal of Economic Entomology 109: 1015–1019.

Toledo-Hernández, R. A., J. Toledo & D. Sánchez. 2018. Effect of *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) on food consumption and mortality in the Mexican fruit fly, *Anastrepha ludens* (Diptera: Tephritidae). International Journal of Tropical Insect Science 38: 254-260.

USDA-APHIS (United States Department of Agriculture, Animal Plant Inspection Service). 2000. Effectiveness of Success 0.02 CB for the Control of Fruit Flies. Internal Report, USDAAPHIS-PPQ, Guatemala City, Guatemala.

Valero-Jiménez, C. A., H. Wiegers, B. J. Zwaan, C. J. M. Koenraadt & J. A.L.van Kanc. 2016. Genes involved in virulence of the entomopathogenic fungus *Beauveria bassiana*. Journal of Invertebrate Pathology 133: 41-49

Vargas, R. I.; R. F. L. Mau, E. B. Jang, R. M. Faust & L. Wong. 2008. The Hawaii Fruit Fly Areawide Pest Management Programme. Publications from USDA-ARS / UNL Faculty. 656. http://digitalcommons.unl.edu/usdaarsfacpub/656

Vega F. E., P. F. Dowd, L. A. Lawrence, J. K. Pell, M. D. Jackson & M. G. Klein. 2000. Dissemination of beneficial microbial agents by insects. pp 153-177 *In*: L.A. Lacey & H. K. Kaya (Eds.) Field Manual of Techniques in Invertebrate Pathology. The Netherlands: Kluwer Academic Publishers.

Vega, F. E, P. F. Dowd, L. A. Lacey, J. K. Pell, D. M. Jackson & M. G. Klein. 2007. Dissemination of beneficial microbial agents by insects, pp 127-146. In: Lacey L. A & Kaya H. K. (Eds.) Field manual of techniques in invertebrate pathology, Second edition. Springer, Dordrecht, The Netherlands.

Weldon, C. W., M. K. Schutze & M. Karsten. 2014. Trapping to Monitor Tephritid Movement: Results, Best Practice, and Assessment of Alternatives, pp 175-217. In: Shelly T., N. Epsky, E. B. Jang, J. Reyes-Flores & R. Vargas (eds.) Trapping and the Detection, Control, and Regulation of Tephritid Fruit Flies, Springer, Dordrecht, The Netherlands.

Wilson, W. M., J. E. Ibarra, A. Oropeza, M. A. Hernández, R. A. Toledo-Hernández & J. Toledo. 2017. Infection of *Anastrepha ludens* (Diptera: Tephritidae) adults during emergence from soil treated with *Beauveria bassiana* under various texture, humidity, and temperature conditions. Florida Entomologist 100: 503-508.

Wraight, S.P., M. A. Jackson & S.L. de Kock. Production, Stabilization and Formulation of Fungal Biocontrol Agents, pp. 253-288. *In*: T.M. Butt, C. Jackson & N. Magan (Eds.) Fungi as Biocontrol Agents: Progress, Problems and Potential. CAB International, London UK.

Yousef, M., M. D. Lozano-Tovar, I. Garrido-Jurado & E. Quesada-Moraga. 2013. Biocontrol of *Bactrocera oleae* (Diptera: Tephritidae) with *Metarhizium brunneum* and its extracts. Journal of Economic Entomology, 106: 1118-1125.

Yousef, M., I. Garrido-Jurado & E. Quesada-Moraga. 2014. One *Metarhizium brunneum* strain, two uses to control *Ceratitis capitata* (Diptera: Tephritidae). Journal of Economic Entomology 107: 1736-1744.

Yousef, M., E. Aranda-Valera & E. Quesada-Moraga. 2018. Lure-and-infect and lure-and-kill devices based on *Metarhizium brunneum* for spotted wing *Drosophila* control. Journal of Pest Science 91: 227–235.

Zimmermann, G. 2007a. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Science and Technology 17: 879-920.

Zimmermann, G. 2007b. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Science and Technology 17: 553-596.

Zimmermann, G. 2008. The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology and use in biological control. Biocontrol Science and Technology 18: 865-901.

Appendix 1. Studies on the use of entomopathogenic fungi for the control of fruit flies.

Fungus	Fly species	Research topics	Country	Reference
B. bassiana	*A. ludens*	Pathogenicity, LT_{50} and LC_{50} against larvae, pupae and adults in laboratory conditions.	Mexico	De la Rosa et al. 2002
B. bassiana	*A. ludens*	Effect of two formulations, on copulation behavior and horizontal transmission	Mexico	Toledo et al. 2007
B. bassiana	*A. ludens*	Effect on the behavior of infected males against uninfected males	Mexico	Novelo-R. et al. 2009
B. bassiana	*A. ludens*	Effect on adult emergence in soil under laboratory conditions.	Mexico	Wilson et al. 2017
B. bassiana	*A. obliqua*	Susceptibility of adults under laboratory conditions	Mexico	Hernández–D–O. et al. 2010
B. bassiana	*A. ludens*	Effect on ovarian development, fecundity and fertility under laboratory conditions.	Mexico	Sánchez-R. et al. 2012
B. bassiana	*A. obliqua*	Horizontal transmission of *Beauveria* conidia on adults of *Anastrepha obliqua* using vectors and disseminator devices: Effect on sexual competitiveness and fecundity.	Mexico	Campos et al. 2014
B. bassiana	*B. carambolae*	Evaluation against larvae with addition of lufenuron under laboratory conditions	Indonesia	Hadi et al. 2013
B. bassiana	*B. carambolae*	Effect of the combination with lufenuron on the reproductive capacity under laboratory conditions.	Indonesia	Ndii et al. 2016
B. bassiana	*B. carambolae*	Combination with lufenuron to reduce reproductive capacity under laboratory conditions.	Indonesia	Hadi et al. 2017
B. bassiana	*Z. cucurbitae* *B. zonata*	Isolation of soil strains and effect on fruit flies	Mauritius	Sookar et al. 2008
B. bassiana	*Z. cucurbitae, B. zonata*	Effect on pupae under laboratory conditions	Mauritius	Sookar et al. 2010
B. bassiana	*B. dorsalis*	Evaluation of entomopathogenic fungi under laboratory conditions	Thailand	Aemprapa 2007

Appendix 1. Studies on the use of entomopathogenic fungi for the control of fruit flies.

Fungus	Fly species	Research topics	Country	Reference
B. bassiana	*B. dorsalis*	Effect of commercial formulation on larvae and adults under field conditions	Ghana	Marri et al. 2016
B. bassiana	*B. oleae*	Effect of two commercial products under laboratory and semi-field conditions	Greece	Anagnou-V. et al, 2005
B. bassiana	*B. oleae* *Z. cucurbitae*	Pathogenicity of strains in wettable powder and wettable granules to fruit flies in laboratory conditions	India	Jiji et al. 2006
B. bassiana	*B. oleae*	Evaluation of commercial products against adults under laboratory conditions	Egypt	Mahmoud 2009a
B. bassiana	*B. oleae,* *C. capitata,* *R. cerasi*	Biopesticide applications in olive trees	Italy	Benuzzi et al. 2007
B. bassiana	*B. zonata*	Susceptibility of larvae, pupae and adults under laboratory conditions	Egypt	Mahmoud 2009b
B. bassiana	*B. zonata*	Evaluation of sterile males as vectors for fungus dispersal to uninfected insects under field conditions.	Mauritius	Sookar et al. 2014a
B. bassiana	*B. zonata*	Effect against larvae and adults under laboratory conditions.	Egypt	Rashad et al. 2015
B. bassiana	*B. zonata*	Pathogenicity against larvae and adults under laboratory conditions.	Pakistan	Gul et al. 2015
B. bassiana	*B. zonata,* *C. capitata*	Effect against larvae and pupae under laboratory conditions.	Egypt	Soliman et al. 2014
B. bassiana	*C. capitata*	Susceptibility of pupae and adults under laboratory conditions	Greece	Beris et al. 2013
B. bassiana	*C. capitata*	Effect against pupae and adults under laboratory conditions	Spain	Quesada-M. et al. 2006
B. bassiana	*C. capitata*	Pathogenicity against pupae in soil under laboratory and greenhouse conditions.	Brazil	Almeida et al. 2007
B. bassiana	*C. capitata*	Effect on adults under laboratory conditions	Costa Rica	Porras & Lecuona 2008
B. bassiana	*C. capitata*	Effect against adults under laboratory conditions	Spain	Medina et al., 2009
B. bassiana	*C. capitata*	Effect against adults under laboratory and field conditions.	Italy	Ortu et al. 2009
B. bassiana	*C. capitata*	Pathogenicity against adults under laboratory conditions	Mexico	Muñoz et al. 2009

Appendix 1. Studies on the use of entomopathogenic fungi for the control of fruit flies.

Fungus	Fly species	Research topics	Country	Reference
B. bassiana	*C. capitata*	Determination of LC_{50} on larvae and pupae under laboratory conditions	Brazil	Queiroz de O. et al. 2010
B. bassiana	*C. capitata*	Horizontal and vertical transmission and effect on fecundity increase and population suppression.	Mexico y Guatemala	Toledo et al. 2014
B. bassiana	*C. capitata*	Evaluation of sterile males as disseminators of conidia under field conditions.	México y Guatemala	Flores et al. 2013
B. bassiana	*C. capitata*	Pathogenicity on larvae under laboratory conditions	Morocco	Imoulan & Elmeziane 2014
B. bassiana	*C. capitata*	Pathogenicity of Indian strains on adults under laboratory conditions.	India	Elbashir et al. 2014
B. bassiana	*C. capitata*	Evaluation on larvae and pupae under laboratory conditions.	Brazil	Bisolli et al. 2014
B. bassiana	*C. capitata*	Effect on pupae under laboratory conditions	Italy	Oreste et al. 2015
B. bassiana	*C. capitata*	Evaluation of native Palestinian strains against adults under laboratory conditions	Palestine	Qazzaz et al. 2015
B. bassiana	*C. capitata*	Molecular characterization and effect of native strains against adults under laboratory conditions	Morocco	Imoulan et al. 2016
B. bassiana	*C. capitata*	Potential of strains obtained in soil samples against larvae, pupae and adults.	Morocco	Hallouti et al. 2017
B. bassiana	*C. capitata*	Effect of three formulations against adults under laboratory conditions and efficacy of disseminator devices under field conditions.	Mexico y Guatemala	Toledo et al. 2017
B. bassiana	*C. capitata, C. cosyra, C. rosa fasciventris*	Effect on pupae and adults emergence under laboratory conditions.	Kenya	Ekesi et al. 2002
B. bassiana	*C. capitata, C. cosyra, C. rosa fasciventris*	Exposition of fruit flies to dry conidia and evaluation in field cages of three autoinoculation devices	Kenya	Dimbi et al. 2003a

Appendix 1. Studies on the use of entomopathogenic fungi for the control of fruit flies.

Fungus	Fly species	Research topics	Country	Reference
B. bassiana	*C. capitata, B. oleae*	Effect on adults by oral and contact under laboratory conditions.	Greece	Konstantopoulou and Mazomenos 2005
B. bassiana	*C. capitata, A. ludens*	Effect of strains against adults under laboratory conditions	Mexico	Toledo et al. 2008
B. bassiana	*C. capitata, C. rosa*	Effect on adults and pupae under laboratory conditions.	South Africa	Goble et al. 2011
B. bassiana	*R. cerasi*	Application of *B. bassiana* as a bioinsecticide under field conditions.	Italy	Ladurner et al. 2008
B. bassiana	*R. cerasi*	Evaluation of two myco-insecticides under field conditions	Italy	Daniel & Wyss 2008
B. bassiana	*R. indifferens*	Susceptibility of larvae and pupae under laboratory conditions	Canada	Cossentine et al. 2010
B. bassiana	*R. pomonella*	Susceptibility of larvae and pupae under laboratory conditions	Mexico	Muñiz-R. et al. 2014
B. bassiana	*Z. cucurbitae*	Sexual competitiveness of infected sterile males under semi-field and field cages conditions	Mauritius	Sookar et al. 2014a
B. pseudobassiana	*C. capitata*	Effect on eggs, larvae, pupae and adults under laboratory conditions.	Italia	Bedin et al. 2018
I. fumosorosea	*A. ludens*	Effect on larvae and pupae under laboratory conditions	Mexico	Gandarilla-P. et al., 2012
M. anisopliae	*A. fraterculus*	Effect on larvae, pupae and adults emergence under laboratory conditions	Brazil	Destéfano et al., 2005
M. anisopliae	*A. ludens*	Virulence on third larvae instar under laboratory conditions and the effect in emergence reduction of *A. ludens* in field cages	Mexico	Lezama-G. et al., 2000
M. anisopliae	*A. ludens*	Effect on consumption and sublethal effects in mortality under laboratory conditions	Mexico	Toledo-H. et al., 2018
M. anisopliae	*A. obliqua*	Selection of strains and the effectiveness against adults in laboratory conditions.	Colombia	Osorio-F. & Canal 2011
M. anisopliae	*B. carambolae*	Effect on larvae, pupae and adults under laboratory conditions.	Brazil	Brito et al., 2019

Appendix 1. Studies on the use of entomopathogenic fungi for the control of fruit flies.

Fungus	Fly species	Research topics	Country	Reference
M. anisopliae	*B. dorsalis*	Ecology and management of native and exotic fruit flies in Africa	Kenya	Ekesi et al., 2016
M. anisopliae	*B. zonata, C. capitata*	Susceptibility of adults under laboratory conditions	Egypt	Ibrahim et al., 2014
M. anisopliae	*Bactrocera spp.*	Effect on pupae under laboratory conditions	Thailand	Mar & Lumyong 2012
M. anisopliae	*C. capitata*	Effect on pupae in soil at controlled temperature and moisture in laboratory conditions	Kenya	Ekesi et al. 2003
M. anisopliae	*C. capitata*	Effect of pesticides in soil on the pathogenicity of fungus, and evaluation of application on soil surface in suspension and dry conidia against larvae	Brazil	Mochi et al. 2006
M. anisopliae	*C. capitata*	Horizontal transmission of conidia under laboratory conditions.	Spain	Quesada-M. et al. 2008
M. anisopliae	*C. capitata*	Susceptibility of larvae and adults under laboratory conditions.	Algeria	Boudjelida & Soltani 2011
M. anisopliae	*C. capitata*	Virulence on sterile flies and attraction to contaminant devices in laboratory conditions	Spain	San Andres et al., 2014
M. anisopliae	*C. capitata*	Efficacy of a contaminant device in field conditions	Spain	Navarro-L. et al. 2015
M. anisopliae	*C. capitata, C. cosyra, C. rosa C. fasciventris*	Effect of specie, age and sex and susceptibility in laboratory	Kenya	Dimbi et al. 2003b
M. anisopliae	*C. capitata, C. cosyra, C. rosa fasciventris*	Effect of constant temperature on germination of conidia and susceptibility to different temperature ranges	Kenya	Dimbi et al. 2004
M. anisopliae	*C. capitata, C. cosyra, C. fasciventris*	Effect on pupae and endoparasitoids in soil	Kenya	Ekesi et al. 2005

Appendix 1. Studies on the use of entomopathogenic fungi for the control of fruit flies.

Fungus	Fly species	Research topics	Country	Reference
M. anisopliae	*C. capitata, C. cosyra, C. fasciventris*	Effect of inoculation on the mating behavior under laboratory conditions	Kenya	Dimbi et al. 2009
M. anisopliae	*C. capitata, C. cosyra, C. fasciventris*	Horizontal transmission, and effect on fecundity and fertility	Kenya	Dimbi et al. 2013
M. anisopliae	*R. cerasi*	Susceptibility on different biological stages under laboratory conditions	Italy	Daniel & Wyss. 2009
M. anisopliae	*B. zonata, Z. cucurbitae*	Effect on fecundity and fertility under laboratory conditions	Mauritius	Sookar et al. 2014b
M.anisopliae	*C. capitata*	Effect on fecundity and fertility under laboratory conditions.	Spain	Castillo et al. 2000
M. brunneum	*B. oleae*	Infection of larvae, pupae and adults and effect of crude extract to adults under laboratory conditions	Spain	Yousef et al. 2013
M. brunneum	*C. capitata*	Evaluation against adults and activity of crude extract under laboratory conditions.	Spain	Yousef et al. 2014
M. brunneum	*C. capitata*	Insecticidal activity of crude extract and metabolites against adults under laboratory conditions	Spain	Lozano-T. et al., 2015
M. brunneum	*R. indifferens*	Susceptibility of larvae and pupae under laboratory conditions	Canada	Cossentine et al. 2011
M. brunneun	*B. oleae*	Evaluation against emergence in soil under laboratory conditions.	Spain	Yousef et al., 2018
M. guizhouense	*Z. cucurbitae*	Effect on sexual competitiveness and mating propensity	Thailand	Thaochan & Ngampongsai 2015
P. lilacinus	*Z. cucurbitae*	Susceptibility of adults under laboratory conditions	India	Amala et al. 2013