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WORKING MATERIAL

Improving Rearing, Handling, and Field Components for Fruit Fly SIT Application

Report of the First Research Coordination Meeting (virtual format) of an FAO/IAEA Coordinated Research Project

1–5 November 2021
Held virtually

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I. BACKGROUND

1.1 Scientific Situation and Problems to be Researched

Fruit flies are one of the most destructive pests affecting production and international trade of fruits and vegetables worldwide. As such, fruit fly pests are a significant constraint in reaching the sustainable development goals of the UN by affecting food security and safety as well as poverty reduction and the environment.

In the past decades the sterile insect technique has been successfully incorporated to the integrated fruit fly management against some of the most important fruit fly pests. SIT has been used for pest exclusion, containment, suppression, and eradication. Examples of successful high impact sterile insect technique (SIT) interventions against fruit fly pests include operational programmes in Argentina, Australia, Chile, Croatia, Guatemala, Israel, Mauritius, Mexico, Peru, Spain, Thailand and the USA.

Nevertheless, technological gaps, lack of harmonization of technologies and tools, and lagging adaption of technological innovations have been observed in operational programmes in Member States. In addition, this environment-friendly technology is continuously competing with conventional pest control methods. This situation can be observed in various components of SIT used against fruit fly pests, including colony management, mass-rearing of insects, sterilization and post-irradiation handling and release. It can also be observed in field components including surveillance systems and population suppression methods. Applied research is required to adopt these technologies and improve cost effectiveness. Optimizing and harmonizing the use of the SIT will further provide comparative advantages to this nuclear based technology.

Targeted species: The following fruit fly species of economic and quarantine importance are considered to be potential targets for the improved SIT and related technologies: *Anastrepha ludens, Anastrepha obliqua, Anastrepha fraterculus, Bactrocera dorsalis, Bactrocera correcta, Bactrocera tryoni, Bactrocera zonata, Ceratitis capitata* and *Zeugodacus cucurbitae*.

1.2 Importance of Mass-rearing in SIT Programmes

The requirements of mass rearing large quantities of high-quality insects at a low cost and ensuring that irradiation processes have a minimum adverse effect on sterile insects was raised by Knipling (1955) from the conception of the SIT. Over the years, in fruit flies, extraordinary advances have been made in the production of millions of sterile insects per week at low costs. These insects have fulfilled their objective, but the improvement of their quality, and particularly, of their sexual competitiveness in the field, continues to represent a challenge. In this CRP the research will focus on improving the performance of genetic sexing strains (GSS) through introducing fresh wild genes into the breeding colonies and on improving mass rearing through novel diets such as gel diets which provide advantages such as optimization of rearing space and disposal of the spend diets. In addition, the possibilities of using new bulking agents in solid diets as a solution to the problem of the lack of stability of their chemical composition will be studied, as well as the incorporation of new nutritional compost to improve the quality of the larvae.

1.3 Importance of Sterile Male Performance and Sterile Fly Release

The release of sterile insects is the last operational step in a series of complex processes that seek to ensure that the appropriate conditions are provided to sterile insects to carry out their function of introducing sterility to wild populations of the pest. One critical factor is the adequate feeding of sterile adult flies before release. Proper feeding including the supply of proteins and carbohydrates, will result in longer lifespan of sterile males and a better mating propensity both vital factors for efficient application of the sterile insect technique (SIT). Developments in packing, holding and sterile insect release have been continuous over the years, yielding a number of technological options that could be considered/implemented in action programmes to increase their operational efficiencies. A decision support tool (or sterile fly optimization model) was recently developed for optimization of sterile fly release. This model is used by programme managers to assess the required sterile fly density in the field based on the sterile to fertile rations being obtained. By adjusting the densities, the use of sterile flies is optimized which has a positive effect on pest suppression and eradication and on the cost-effectiveness of the technology. The success in implementing adequate practices also secures the large investment made during the production and maintenance of mass reared strains.

1.4 Importance of Trapping Systems and Control Methods

Trapping is a key component of programs against Tephritids, especially when there is an effective lure available. For a preventative SIT program such as the one operated in California USA, surveillance relies on trap networks for detection of incursions. In situations where the targeted pest is established or endemic, trap networks give general information on seasonal abundance and spatial distribution and so can be helpful for setting release rates and locations for effective SIT (Barclay et al 2016).

To reduce the populations of these pests, mixtures of protein or food attractant with chemical products have traditionally been used. Organophosphate products have been part of the molecules used as pesticides. Currently, there are alternatives in the form of bait stations that have demonstrated to reduce the populations of several species of fruit flies in addition to being compatible with organic production. It has also been documented that it does not have environmental consequences including the avoidance of damage to pollinators, invaluable for agriculture.

II. CO-ORDINATED RESEARCH PROJECT (CRP)

This Coordinated Research Project (CRP) is based on a Consultants' Meeting that was held virtually from 7–11 June 2021 to assess the potential for conducting co-ordinated R&D on improving the sterile insect technique (SIT), and to formulate a proposal for a CRP on "Improving rearing, handling, and field components for fruit fly SIT application".

The overall objective of this new CRP D41029, "Improving rearing, handling, and field components for fruit fly SIT application "approved for the period 2021–2025, is to further optimize and harmonize through applied research the use of the SIT and related technologies for management of plant pests.

III. FIRST RESEARCH CO-ORDINATION MEETING (RCM)

The first RCM was held virtually from 1–5 November 2021. The list of participants, which included 19 CRP contract and agreement holders from 13 countries, as well as 60 additional observers, is given in Annex 1. The agenda for the meeting is attached in Annex 2.

During the first two days of the meeting RCM participants presented research relevant to the CRP, as well as their research plans for the first 18 months of the CRP.

During the last three days of the meeting, general discussions were held to define and review the thematic areas of the CRP (Table 1) and to review the general and specific R&D objectives to be addressed during the 5 years of the CRP and the CRP Logical Framework, in order to agree on minimum outputs to be achieved at the end of the CRP. Furthermore, participants were divided into three working groups (Annex 3) to develop more detailed R&D plans to be conducted during the first 18 months of the CRP. Chief Scientific Investigators (CSI's) were grouped according to the research topic of interest as shown in Table 2.

Abstracts of the presentations are presented in Annex 4 and a copy of all PowerPoint presentations is available to all participants in the TEAMS group specially created for this RCM.

Table 1. Thematic areas being addressed by researchers.

TOPIC	SUBTOPIC	ADVANTAGE OF INNOVATION
1. Production (Mass-rearing)	1.1 Colony management and GSS strains	1.1.1 Maintaining high genetic diversity through a novel genetic model and through genetic sexing strains
	1.2 Diets (liquid, solid and gel)	1.2.1 Advantages in space and waste management
2. Post-Production (Packing and holding)	2.1 Supplements including aromatherapy and protein based adult food	2.1.1. Supplements to enhance sterile male performance.
	2.2 Sterile fly release model (decision support tool)	2.2.1 Excel model for sterile fly release optimization
3. Field Operations (Surveillance systems and control methods)	3.1 Optimization of surveillance systems	3.1.1 Improved trapping systems (lures, traps and risk-based models)
	3.2 Control methods	3.2.1 Improved sterile fly release through decision making tools such as density models 3.2.2 Improve fruit fly suppression through validation and harmonization of bait stations for mass trapping of adult flies.

Table 2. Chief Scientific Investigators (CSI's) grouped by topic and subtopics (see Table 1) of interest.

CONTRACT AND AGREEMENT HOLDERS	TITLE PROPOSAL	TOPIC		
		1 PRODUCTION (GSS + Diets)	**2 POST-PRODUCTION (Supplements + SF Release)**	**3 FIELD OPERATIONS (Traps + BS)**
1. David Haymer USA	Introduction of Wild Genetic Material in Breeding Colonies of Genetic Sexing Strains for Maintenance of High Levels of Genetic Diversity and Improvement of SIT	1.1 *Ceratitis capitata* and *Anastrepha ludens*		
2. Dori Nava Brazil	Use of a gelling/texturing agent to replace agar in artificial diet for *Anastrepha fraterculus* larvae, aiming at the sterile insect technique and biological control	1.2 *A. fraterculus*		
3. Diego Segura Argentina	Improving the field performance of *Anastrepha fraterculus* sterile males through specific refreshing protocols and pre-release treatments	1.1 *A. fraterculus*	2.1 *A. fraterculus*	
4. Valter Arthur Brazil	Development and evaluation of genetic sexing strains for *Anastrepha fraterculus* to enable sterile male-only releases in Brazil	1.1 *A. fraterculus*		
5. Carlos Pascacio-Villafán Mexico	Development and Optimization of Gel Diet Rearing Systems for Improving the Sterile Insect Technique Against *Anastrepha ludens* and Ceratitis capitata	1.2 *A. ludens* and *C. capitata*		
6. Cristian Morales Guatemala	Studies in Biofactories on Nutritional Larval Diets and Development and Maintenance of Genetic Sexing Strains of Two Species of Fruit Flies of Economic Importance	1.1 *A. ludens* & C. capitata. 1.2 *C. capitata*		

7. Cristopher Weldon South Africa	Improvements for rearing and performance of sterile fruit flies through manipulation of dietary lipids	1.2 *B. dorsalis*		
8. Preeaduth Sookar Mauritius	Improving rearing and control techniques with the integrated use of SIT for *B. dorsalis*, *B. zonata* and *Z. cucurbitae*	1.2 *B. dorsalis, B. zonata* and *Z. cucurbitae*	2.2 *B. dorsalis, B. zonata* and *Z. cucurbitae*	3.1 & 3.2 *B. dorsalis, B. zonata* and *Z. cucurbitae*
9. Mariel Vanin - Argentina	Improving SIT and field components - GSS strains and gel diets / Improved trapping	1.1 & 1.2 *C. capitata*		3.1 *C. capitata*
10. Polychronis Rempoulakis Australia	Optimize fruit fly production and rear out systems, improving fruit fly management practices, enhance fruit surveillance and control by introducing improved trapping systems and decision-making tools for management of trapping networks		2.1 *Bactrocera tryoni*	3.1 3.2 *B. tryoni*
11. Thi Kim Lien HA Viet Nam	Influence of Dietary Protein on Performance of Sterile *Bactrocera dorsalis* and *Bactrocera correcta* male		2.1 *Bactrocera dorsalis, B. correcta*	
12. Marta Martinez Spain	Development of new technologies to improve rearing, handling, monitoring and release systems in fruit fly SIT programmes	1.1 & 1.2 *C. capitata*	2.1 & 2.2 *C. capitata*	3.1 *C. capitata*
13. David Nestel Israel	Prototype for a Fruit Fly Decision Making System based on Electronic Traps			3.1 *C. capitata*
14. Karim Nebie Burkina Faso	Development of "attract and kill" tools and analyzing SIT possibilities for fruit fly sustainable management in Burkina Faso			3.2 *B. dorsalis, C. cosyra*
15. Julio Cesara Rojas Leon Mexico	Development and optimization of infochemical-derived			3.1 *A ludens, A obliqua*

	lures for monitoring *Anastrepha* fruit flies			
16. Katharina Merkel Australia	Strengthen South Australia's fruit fly response program through a model-based adaptive management tool and targeted applied research	1.2 *B. tryoni*	2.1 *B. tryoni* *C. capitata*	3.1 *B. tryoni* *C. capitata*
17. Bishwo Mainali Australia	Enhancing fruit fly sterile insect technique through improved and cost-effective gel larval diet, pre-release handling, and monitoring	1.2 *B. tryoni* *C. capitata*	2.1 *B. tryoni* *C. capitata*	3.1 *B. tryoni* *C. capitata*
18. Nicholas Manoukis USA	Use of the TrapGrid Computer Model to Optimize Trapping Networks			3.1 *C. capitat*a, *B. tryoni*, *Anastrepha ludens* and others depending on interest
19. José Esteban Santiago Mexico	Improving rearing, handling and field components for fruit fly SIT applications	1.1 *C. capitata*	1.2 *C. capitata*	

The thematic areas were grouped into three mayor categories: 1) Production Process, 2) Postproduction Process and 3) Field operations. Each category with specific research topics as presented in Table 1.

IV. DESCRIPTION OF RESEARCH TOPICS AND METHODOLOGIES

4.1 PRODUCTION PROCESS

4.1.1 Genetic Sexing Colonies

Participants: Carlos Caceres, Cristian Morales/Edwin Ramirez, David Haymer, Diego Segura, Jose Santiago /Salvador Meza*, Mariel Vanin, Pablo Liedo, Valter Arthur/Thiago Mastrangelo*

Background Situation Analysis

Improving breeding colonies by maintaining genetic diversity

Currently, there is no standard protocol to introduce and maintain genetic variability in the breeding colony. Newly develop genetic sexing system will allow for the creative possibility to cross in mass numbers of females which typically carry specific phenotypes used as markers. By carefully following an appropriate cross scheme, the sexing characteristics can be properly preserved while allowing, in parallel, the direct injection of wild material into the breeding colony. By precisely repeating this as a continuous process, the breeding colony will properly maintain its genetic diversity. It is realistically expected that those sterile insects with a high degree of genetic diversity will be more competitive when released in the field.

However, as these assumptions have not been properly validated, one of the objectives of the CRP is to validate the protocols for introgression of new genetic material into the filter colony then validate the production and quality profile of the resultant offspring, including mating competitiveness, in working field cages. Validation and quantification of the introgression should be done by using appropriate genetic protocols and tools that allow for the demonstration of a correlation between genetic diversity and the quality of the insects.

Current Knowledge

Sterile insects in the field need to move, survive, and maintain their mating behaviour to compete for mating with fertile wild males in target areas subject to control. Insect colonization could affect those specific patterns and behaviour since laboratory colonies are maintained in different artificial environments. Insects adapted to laboratories conditions as other domesticated organisms lose their heterozygosity in a few generations. The mating behaviours of tropical fruit flies present very complex courtship behaviours with males aggregating in mating arenas or leks, and where receptive females determine mate choice (Hendrichs et al. 2002; Robinson et al. 2002). Thus, special attention in terms of product quality control must be given to the effects of colonization (Cayol 2000; Hendrichs et al. 2002). For example, the olive fly changed drastically its genetic composition in the first 5 generations of adaptation under laboratory conditions (Zygouridis et al, DOI:10.1111/jen.12042).

Hybridization of the laboratory with wild insects and the selection process for specific traits has demonstrated that the insect could preserve specific characteristics during several generations (McInnis et al 2002), however, that characteristics are not permanent since the selection process under the laboratory conditions will force the colony to back to have again a high degree of homozygosity. A direct correlation between increasing numbers of generations in laboratory culture and the loss of such genetic diversity was also shown for the melon fly (Haymer 1992). The maintenance of mass reared colonies of insects from genetic sexing and other mass reared strains used for application of the sterile insect technique (SIT) also present many other challenges. The first goals of any mass rearing may include resolving the logistics of producing massive numbers of individuals. Beyond this, however, rearing experts have long been aware of the need to pay attention to quality issues to ensure that the released mass reared insects can survive and be competitive with their natural counterparts for SIT to be effective. These quality issues include maintenance of high levels of genetic variation to reduce the impact of intense selection and inbreeding effects inherent in any laboratory rearing environment, but also the inclusion of desirable traits related to mating behaviour and survival of the flies to enhance their effectiveness in SIT programmes. To accomplish this, methods are required to monitor and maintain high levels of genetic diversity in genetic sexing and other mass reared strains and to improve the quality of the flies produced. Specific genetic markers should be used for the controlled monitoring of the introduction of desirable genes and other new genetic material into the colonies to enhance genetic variation in general and for improved mating performance and other aspects of competitiveness in the sterile flies to be released for SIT.

Gaps Identified

Tools for monitoring and addressing the loss of genetic variation and desirable behavioural phenotypes inherent in the establishment of new mass rearing strains are currently inadequate. These issues are often compounded when genetic sexing strains (GSS) requiring filter rearing systems are incorporated into the mass rearing process.

The current configuration of the genetic sexing system based on T (Y ; A) translocations complicates the genetic refreshment of the breading colony at fruit flies' mass-rearing facilities.

The lack of appropriate crossing scheme to introduce wild genetic material into GSS strains.

The lack of evidence that the reduction of heterozygosity reduces fitness and mating competitiveness in the field.

New genetic markers derived from specific genes underlying desirable behavioural phenotypes and anonymous genetic markers distributed around the genome must be identified and mapped according to their chromosome location.

Techniques of marker assisted selection should be implemented to guide the incorporation of specific genetic markers and other segments of the genome into mass reared strains to improve levels of genetic variation and performance of flies produced by the mass rearing process.

Standard Protocol for Colony Refreshment

Protocols for the introduction of wild material into the breeding should meet some logical requirements:

- Avoid single pairs
- Allow for the direct and continues crossbreeding of wild genetic material to avoid again losing genetic diversity during the amplification process and between generations

For sexing strains based on T (Y ; A) translocations, the refreshment with wild material is difficult, since the target individuals that should be crossed with wild insects must be the lab-adapted females which carry in homozygous conditions the alleles used as markers (e.g. wp and tsl of bp). As wild males do not carry the translocation, there is no way to initiate a direct crossing. Therefore, is strongly recommended to use the new sexing mechanisms recently developed at IPCL which the females carry the markers translocated to the X chromosomes. If the females carry a homozygous T (X ; A) translocation, then they can be mass-crossed with wild-type males to facilitate introduction of new genetic material.

For medfly, there are already 2 strains based on such a mechanism currently under mass-rearing evaluation. But small-scale experiments have shown that its production and quality control profile is similar or superior to the conventional Vienna-8 strain based on a T (Y; A) translocation (Caceres et at in preparation).

For other fruit fly species for which SIT programmes currently exist in operation, such as *Anastrepha ludens*, this type of strain still should be developed. The protocol can be provided by IPCL staff or simply replicated from the oncoming publication in which the protocols of isolation are described (Caceres et at in preparation).

4.1.2 Artificial Rearing

Participants: Bishwo Mainali*, Carlos Pascacio*, Chris Weldon, Christian Morales/Edwin Ramirez, Dori Nava, Mariel Vanin, Martha Martinez/Ignacio Pla, Preeaduth Sookar, Valter Arthur/Thiago Mastrangelo, Salvador Meza/Emilio Hernandez, Katharina Merkel.

Background Situation Analysis

Improved Larval Diets

Mass-rearing facilities around the world that produce tephritid fruit flies for use in the Sterile Insect Technique (SIT), share the common need to constantly improve rearing processes to produce the largest numbers of insects of the highest quality at the lowest possible cost (Orozco-Dávila et al. 2017; Mumford 2021; Parker et al. 2021). Artificial diets are key elements for the successful application of the SIT as they permit a constant production of the millions of flies that are sterilized and released in the field (Parker et al. 2021). More importantly, the larval diet is a strong predictor of many functional traits of flies that are critical to ensure that sterile males live long enough in the field until they can copulate with wild females (Orozco-Dávila et al. 2017; Lance & McInnis 2021; Parker et al. 2021). As such, it should come as no surprise that artificial diet development and optimization of rearing processes are key topics of research to further advance the SIT against tephritid pests (Cáceres et al. 2014; Moadeli et al. 2017; Pascacio-Villafán et al. 2017, 2020; Aceituno-Medina et al. 2020; Mastrangelo et al. 2021; Bourtzis & Vreysen 2021).

The search for new diet formulations and new ingredients that improve the cost-effective production of hundreds of millions to billions of sterile flies for use in SIT releases, is a priority in mass-rearing facilities seeking the continuous improvement of their processes (Orozco-Dávila et al. 2017). Solid diets that incorporate bulking agents present problems of variable quality and waste disposal. Liquid and gel diets have emerged as promising alternatives, but these also suffer drawbacks, including separation of components, fermentation and need for cleaning and regular replacement of substrates that support developing larvae.

The procedure for using the liquid diet was developed in Hawaii (Chang et al. 2004, 2006 and 2007). This technology was successfully transferred to different countries for trials for the rearing of several fruit fly species in 2009. So far very few countries have adopted the liquid diet for the mass rearing of fruit flies. Hinderances for the adoption of the liquid diet should be overcome so that it can be adopted in mass rearing facilities across the world. For the Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), two promising liquid diet formulations were improved by incorporating agar (0, 0.25, 0.5, 1.0 and 1.5%) to create semiliquid (gel) diets that maintain consistent composition, suppress fermentation, negate the need for supporting substrates and minimize waste. Overall, gel diets containing greater than 0.5% agar outperformed liquid diets (0% agar) and semiliquid diets (0.25% agar) of identical nutritional composition, especially in terms of development rate and productivity (Moadeli et al. 2017). Gel diets showed great promise for rearing of Q-fly and were adopted for use in a mass rearing facility, overcoming many of the constraints of both traditional solid diets and more recently developed liquid diets.

With a basic gel diet available, further investigation emphasised improvements in fly performance by selecting yeast, which is an important protein source of the larval diet. Yeast products, apart from amino acids (protein), contribute carbohydrate, fat and micronutrients, but there can be substantial variation in

the nutritional composition and suitability of yeast products for use in larval diets. Gel larval diets have recently been developed for large-scale rearing of Queensland fruit fly for SIT, and composition of these diets requires optimization for both performance and cost, including choice of yeast products. The team (Moadeli et al. 2018a) investigated different yeasts 1) debittered brewer's yeast (Lallemand LBI2240), 2) hydrolysed yeast (Lallemand FNILS65), 3) inactivated brewer's yeast (Lallemand LBI2250) and 4) inactivated torula yeast (Lallemand 2160-50), including blends (Moadeli et al. 2018 b). The debittered brewer's yeast, a cheaper and readily available yeast, performed as good as inactivated brewer's yeast and significantly better than Torula yeast and yeast hydrolysate (Moadeli et al. 2018 a). The team then investigated if the concentration of wheat germ oil (WGO), a lipid source and one of the most expensive ingredients can be reduced without affecting the fly quality. They reported that the diets containing WGO obviously outperformed diets without WGO, and the concentrations of 0.11% and 0.15% and above provided full benefit in gel diet 2006, 2009 (original formulation 0.15%, 1%, respectively, Moadeli et.al. 2018a). Savings can be made in gel diets without compromising productivity by reducing WGO concentration. Following this, they then investigated different oils to find if an alternative to WGO can be found without compromising fly quality. Canola oil stood out and performed as good as WGO compared to rice bran and sunflower oil. Canola oil is a cheaper option and inclusion of canola oil substantially reduces diet production cost (Moadeli et al. 2018c). While there is a clear need for the inclusion of oils in gel diets, it is clear from the preceding account that cost has been the main impetus for their selection rather than those optimal for fruit fly fitness. A better understanding of the lipid found in fruit flies and how their variation affects performance is needed to establish the best outcomes relative to cost.

Gel diets offer a rearing solution for Queensland fruit fly that eliminates biological bulking agents and yields faster and more synchronous larval development without compromising productivity or quality. The gel diet was tested against the conventionally used solid larval diets, carrot and lucerne chaff diets and it was reported that the gel diet was as good as or better than the solid diets. The gel and carrot diets produced less waste than lucerne chaff diet (Mainali et al. 2019). The sterile Queensland fruit fly factory located at Port Augusta South Australia is perhaps the only factory that uses gel diet for the production of tephritid fruit fly.

Further to the above, artificial larval diets are known to affect the microbiome of Queensland fruit flies, which may in turn impact fly performance (Majumder et al. 2020). In a recent study, high-throughput Illumina sequencing was used to assess the Queensland fruit fly microbiome in colonies reared, for five generations from nature, on two common artificial diets (carrot and gel).

In Mauritius, the artificial larval diet for *Bactrocera dorsalis*, *B. zonata* and *Zeugodacus cucurbitae* is composed of sugarcane bagasse (6%), ground maize (6%), cane sugar (11%), waste brewery yeast (6%), wheat bran (6%), benzoic acid (0.1%), nipagin (0.1%), hydrochloric acid (0.008%) and water (64.8%). Good quality flies are produced in the newly constructed fruit fly rearing facility with percentage egg hatch, percentage emergence and percentage fliers above 77, 85 and 82, respectively. Trials have shown that both the liquid and gel diets can be successfully used for the rearing of the three fruit fly species. The main constraint of the conventional larval diet is the varying quality of the bulking agent (sugarcane bagasse) and the waste brewery yeast. Furthermore, there is a need for bulk storage and waste management. To solve these problems, further studies should be carried out on the liquid and gel diets so that they could be used in the mass rearing of the flies. Endosymbionts (gut-associated bacteria) could be incorporated in larval diet to improve the fruit fly quality.

Current Knowledge

The mass rearing processes we know today for the production of tephritid fruit flies for use in SIT, are based on a long history of scientific research and technological advancements (Aceituno-Medina & Hernández 2020). More than 70 years ago, the first artificial diets that were used for successful rearing of tephritid fruit flies were gel diets with agar as a gelling agent (Marucci & Clancy 1950). In addition to gel diet formulations (Rivera et al. 2007; Pašková 2007; Moadeli et al. 2017; Pascacio-Villafán et al. 2020), there are currently other types of diets for rearing tephritid fruit flies including complex formulations made of chemically defined ingredients (Chang 2004), liquid diets that require an inert solid material to act as a support matrix for feeding larvae (Chang 2006, 2007, 2009; Ekesi et al. 2014; Anato et al. 2017; Pascacio-Villafán et al. 2018), novel pelleted diet formulations (Aceituno-Medina et al. 2020), and the traditional diets with a bulking agent that are most widely used for mass rearing (Hernández et al. 2014).

Despite effective use over many decades of artificial diets with bulking agents for mass rearing tephritid fruit flies, there is a generalized concern among artificial rearing professionals and managers in mass rearing facilities, because the quality of many types of bulking agents is not stable and they can be contaminated (e.g., with mycotoxins) resulting in a drastic reduction of insect production (Cáceres et al. 2014; Aceituno-Medina et al. 2016, 2019). In addition, pollution generated by large amounts of diet waste is also a cause of concern and the goal of near zero diet waste is highly desirable by the SIT industry (Parker et al. 2021).

Liquid diets emerged as an alternative that sought to address the problems associated with bulking agents (Chang 2006, 2007, 2009). However, liquid diets also have disadvantages that limit their use for large scale rearing including separation of components, fermentation, the need for cleaning and regular replacement of costly substrates that support developing larvae (Moadeli et al. 2017; Pascacio-Villafán et al. 2018).

Faced with this situation, interest has returned to the origins of fruit fly artificial diet research and development (Marucci & Clancy 1950), and agar was tested as a gelling agent in the diet of species of economic importance such as *Anastrepha ludens* (Rivera et al. 2007), *Ceratitis capitata* (Pašková 2007) and *Bactrocera tryoni* (Moadeli et al. 2017). But only in the case of *B. tryoni* has the gel diet system been implemented for mass rearing (Crisp et al. 2018).

In addition to agar, other gelling agents that have been tested in tephritid gel diets are carrageenan, gelatin, pregelatinized starch (Rivera et al. 2012; Pascacio-Villafán et al. 2020; Mastrangelo et al. 2021). Significant research efforts have also been made to search for better-quality bulking agents. Mass rearing facilities have always looked for local products that are affordable, of stable quality and can produce high quality insects. This fact has led to the development of different larval diets, as different bulking agents can be found in the countries where facilities are located. Sugar cane bagasse, corn cob, sugar beet pellets, wheat bran, fodder, lucerne chaff, among others have been used to rear different fruit fly species in different countries.

In artificial mass-rearing of tephritid flies, attention should be paid to all the factors affecting production, quality and cost (Orozco-Dávila et al. 2017; Parker et al. 2021). The multifactorial nature of tephritid artificial rearing systems makes it necessary that the development and optimization of diets and processes are also approached from a multifactorial perspective. One effective strategy of experimentation and statistical modelling used by artificial diet and insect rearing specialist for the development of diets and discovery of optimal rearing conditions are statistical Design of Experiments (DOE) and Response Surface Methods (RSM) (Lapointe et al. 2008; Damiens et al. 2012; Cohen 2018; Huynh et al. 2019; Hickin et al. 2021). This strategy of experimentation and modelling approach, has been used to model the cost-effectiveness of fruit fly rearing on artificial diet (Pascacio-Villafán et al. 2017) and for the development of gel diet formulations (Pascacio-Villafán et al. 2020).

Gaps Identified

- Some gelling agents (e.g., agar) are costly, limiting their use for mass rearing.
- In the case of gelling agents that need to be heat-activated (e.g., agar and carrageenan), new cost-effective and sustainable technology is required to prepare gel diets at the factory level
- Evaluation of novel gelling agents is needed.
- Limited use of formal optimization methods applied to artificial diet research and development.
- Lack of information on the interaction of the different components of the diet (lipids, yeasts) and the gelling agent.
- Lack of information on the physical and texture properties of gels that make them suitable for efficient rearing of tephritid larvae.
- There are no specific procedures for the development of gel diets (difficulties in achieving the right consistency).
- Solid diet waste at the factory level is a source of pollution and increases labour work
- Reuse of the waste generated by solid diets is limited. It is necessary to find options for use (e.g. composting).
- Some bulking agents used for mass rearing lack stable quality. The presence of substances that cause a drop in production (e.g. phytosanitary residues) is an issue.

Note from Carlos Pascacio: Dear colleagues, if anyone is interested in applying Design of Experiments and Response Surface Methods to their diet development/optimization problem, I will be happy to collaborate with you.

4.2 POSTPRODUCTION PROCESS

4.2.1 Improved Release Technology (Sterile Fly Densities)

Participants: Katharina Merkel, Mariel Vanin, Martha Martinez/Ignacio Pla*, Pedro Rendon*, Preeaduth Sookar.

Background Situation Analysis

Sterile insect releases are an important activity of SIT programmes. An efficient system is essential to release the best quality of sterile insects possible in order to achieve the final objectives of an operational programme. This system requires the proper handling of the flies after irradiation. The processes involved include insect emergence, holding adult feeding, sexual preconditioning and the actual field release. These processes are an expensive part of an SIT programme, if all or parts of these processes fail, the previous insect production and shipping investments in which the programmes have incurred up to this point are lost.

For this reason, it is extremely important to monitor insect quality and production processes that ensure that the emergence and release of the adults is successful, and it is reliably conducted under the proper set of conditions.

The actual release of sterile insects includes the process of loading/holding insects inside release containers (bags, release boxes or others) until they are delivered into the field.

Sterile insects (sterile males for species where genetic sexing and production of male-only is available) may be released by aerial (aircraft) or ground means. The aerial release process includes filling the release boxes, transporting the flies to the airstrip, loading them into the aircraft, and flying the aircraft to the point where the entire load is released. The aerial release is more effective for large-scale operational programmes, not only economically but also from a technical standpoint, since it achieves better uniformity in the distribution of sterile flies. Ground releases are more suitable for small areas and maybe to complement the aerial releases in wild fruit fly hotspots.

Current Knowledge

Operational programmes using the sterile insect technique as a method for fruit fly management may use aerial or ground releases.

Ground release methods are useful in certain circumstances, such as when the target area of the SIT programme is small or when a spot treatment is desired. It is also an alternative method to aerial releases when aerial releases cannot be carried out due to inclement weather.

Regarding aerial releases, considering all the SIT programmes existing, there are two methodologies for the implementation of aerial releases, 1.) bag release system and 2.) chilled adult release system.

Concerning bag releases, pupae are kept inside paper bags until they emerge and reach sexual maturity. During the flight, flies are released when the bags are torn by the action of mechanical elements (hooks or blades) located at the end of the aircraft's exit ramp. The major advantage of this system is the low equipment requirements. In addition, since the flies are not chilled before release, the loss of fly quality associated with the fly chilling is avoided.

The chilled adult technique release is a system that allows the release of large quantities of flies at the same time, so its use is recommended in large-scale SIT programmes. The main advantage, besides the possibility of releasing large numbers of flies, is that no residues are generated during releases so it is environmentally friendly. Additionally, it is a method with lower labour requirements and decreases the probability of predation.

All aerial release machines are based on a system for maintaining proper temperature and humidity, a fly dosing system a release mechanism and a geographical location system that allows releases to be located and the release activity even documented in printed maps. There are currently three systems for the aerial release of sterile insects: 1.) USDA release system, (2) Mubarqui Smart release machine (México) and (3) dosed release system (Spain).

The USDA system was the first release machine model to be designed. It was based on a funnel which led the flies to a movable belt to be finally released through a chute. Due to the problems with this system, such as low capacity or loss of fly quality due to agglutination, the model has been modified until the current one, which replaces the conveyor belt by screw augers.

The smart aerial release machine is a design of the Mexican company Mubarqui, based on the use of vibratory conveyors. The release speed is programmed and controlled as required by different vibratory feed intensities without damaging the biological material. The machine is controlled via Bluetooth by a tablet with Android operating system including a fully automatic guidance and navigation system (MaxNav and AGNAV software). The tablet is also connected to a database that facilitates the preparation of flight schedules and the automatic storage of flight reports. This system achieves good homogeneity of dispersion.

The release system used in Spain was designed by TRAGSA in collaboration with the "Instituto Valenciano de Investigaciones Agrarias" (Pla et al. 2021). In this system flies are released by means of an auger and allows a variable amount of flies to be released according to the needs previously marked for each of the zones in the area of action (dosed release).

The table shows the release systems used in the different fruit fly SIT programmes.

Fruit Fly Species	Type of Machine	Type of Aircraft	Capacity	Programme
C. capitata	Paper bags	CESSNA 172	3 Million	Argentina
C. capitata	Chilled release machine	TECNAM 206 CESSNA 182	12 Million 12 Million	Argentina
C. capitata	Paper bags	CESSNA 172	--	Chile
C. capitata	USDA	LET 410 UVE	60 Million	Guatemala
C. capitata	USDA	CESSNA 207	2.5-3.5 Million	USA
C. capitata	Chilled release machine	BEECHRAFT KING AIR 90	10 Million	Portugal
C. capitata	Chilled release machine	NORMA ISLANDER	5 Million	Israel
C. capitata	Chilled release machine	CESSNA 207	5 Million	South Africa
C. capitata	Chilled release machine	CESSNA 206	10 Million	Valencia, Spain
C. capitata	MSRM	CESSNA 401 & 402	60 Million	Mexico
A.ludens	MSRM	CESSNA 206	7 Million	Mexico
A.obliqua	MSRM	CESSNA 206	7 Million	Mexico

Source: *FAO/IAEA. 2017. Guideline for packing, chipping, holding and release of sterile flies in area-wide fruit fly control programmes.*

Gaps Identified

Considering terrestrial release methods, the main disadvantage is that it is costly in terms of time and labour and that the area covered is much smaller and is therefore not applicable in AW-IPM programmes. In addition, the distribution of released males is not homogeneous and waste is produced during the process.

Referring to the aerial releases with paper bag, the main disadvantages s the waste they generate, which makes them little environmentally friendly. Moreover, flies can be damaged in the bags because of the limited space in the planes, there is a higher risk of predation as the bags do not open or open only partially once released and finally, the distribution of the flies is not as uniform as desirable. In addition to the above, the preparation, transport, handling, etc. of the necessary bags is labour-intensive, making it a very costly system. Moreover, a operator is required to be at the airplane during the flight to open and release the bags. The number of sterile flies to be released is also much lower than in adult chilled systems.

The main disadvantage of the chilled adults systems is that they have a complex design, which implies the cost of the system itself and the cost of the legal permits for the aircrafts that are going to be modified for their use. However, this method remains the most cost-effective. However, the chilling process of the sterile flies can lead to a decrease in quality parameters, including especially if very long chilling times are required due to the distance between the release facilities and the release areas. In any case,

the process conditions of the cooling process can be controlled to minimize the loss of quality of the adults to be released

A disadvantage to be taken into account in this type of systems is that they are usually designed for a specific model of aircraft. This can lead to a price increase if this model has a low availability or if there is a lack of competition among aircraft companies.

Standard Sterile Fly Release Model

A sterile fly release model was produced by the Moscamed Programme in Guatemala and edited and published by the FAO/IAEA Programme. It is being used in programme operations since 2012. As a result, the release of sterile flies has been optimized increasing the efficiency of SIT and saving a substantial amount of financial resources. This model could be applied and validated for specific fruit fly species and under different environmental conditions as a support decision tool for programme managers.

The model's user's manual and Excel spread sheet can be found in:

https://www.iaea.org/resources/manual/manual-and-spreadsheet-for-assessment-of-sterile-insect-release-densities.

4.2.2 Enhance field performance of sterile males by extending pre-release period, providing food and hormonal supplement, and exposure to/use of semiochemicals before field releases

Participant: Bishwo Mainali, Diego Segura*, Martha Martinez/Ignacio Pla, N.T.T. Hien / H.T.K. Lien/Mariel Vanin, Polychronis Rempoulakis

Background Situation Analysis

The SIT relies on the quality of laboratory reared, sterile males to survive under field conditions and sterilize wild females. Laboratory colonies usually experience genetic processes that reduce the performance of sterile insects when they are released in the field. Sterilization, through ionizing irradiation, sometimes contributes to a further reduction of the biological quality of sterile males. While much research effort has been invested in improving mass-rearing and quality-control procedures at the fly-factory level, the post-factory handling of sterile flies has received much less attention. However, research (conducted mainly from 2000 onwards) has focussed on developing and validating ways of improving sterile male performance through better management during a critical period (starting with the arrival of pupae at the fly emergence and release facility and ending with the release of the sterile flies in the field). This period opens a window of opportunity to provide flies with supplements that improve their performance.

Exposure of sterile males to nutritional, hormonal, and semiochemical treatments has been assessed for improvement of sterile male performance. Likewise, enhancement of post-factory handling and release methods have been also explored. Incorporation of protein and juvenile hormone into pre-release diets significantly accelerates sterile male maturation and improves sexual performance in several species. Use of semiochemical treatments like ginger root oil (GRO) or citrus oils in *Ceratitis capitata*, and methyl eugenol and raspberry ketone in *Bactrocera* and *Zeugodacus* species, significantly increase sterile male mating competitiveness. Some of these supplements have been already adopted as part of

the sterile flies release protocols by several action programmes, mainly the use of GRO for *C. capitata,* however there are many programmes that have been not able to incorporate these innovations due to practical or technical reasons, which points out that research is still needed.

Current Knowledge

As part of a previous FAO/IAEA Coordinated Research Project (CRP) on "Improving Sterile Male Performance in Fruit Fly SIT Programmes" research focused on treatments than could be apply to sterile flies after emergence and before release to improve the field performance and increase SIT efficiency. Research extended beyond the CRP and a significant amount of knowledge accumulated in the fields of applying nutritional, hormonal, and semiochemical treatments. Several operational SIT programmes around the world adopted one or more of these supplements as part of their strategy to increase sterile male success.

Nutrition. Most Tephritidae fruit flies need to forage for protein and nitrogenous compounds in order to mature their reproductive systems (Hendrichs and Prokopy 1994). Sufficient knowledge has been obtained for the genera *Anastrepha, Bactrocera, Ceratitis* and *Zeugodacus* to suggest that yeast hydrolysate can enhance male sexual performance (Kaspi and Yuval 2000, Aluja et al. 2001, Pérez-Staples et al. 2007, Haq et al. 2010a, 2014a). Even though protein seems to have positive effects in most species studied so far, in Ceratitis there seems to be other factors that balance this effect, such as reductions of male survival and dispersal. This adverse effect was reduced when the protein ratio in the diet was lowered. For many species, this potential trade-offs between reproduction and survival have not been addressed (Blay and Yuval 1997; Shelly and Kennelly 2003; Shelly and McInnis 2003; Prabhu et al. 2008; FAO/IAEA 2017, see Pereira et al. 2021).

Despite the benefits associated to the addition on protein to the adult diet, most fly emergence and release facilities do not include nitrogenous compounds in the pre-release diet, and sterile males are generally provided only sugar (Pereira et al. 2021). Studies on more cost-effective protein sources, optimal dosage and delivery in an operational context will surely contribute to the use of protein supplement in SIT programmes (Pereira et al. 2021). The formulation and testing of optimal pre-release diets, containing sugar and protein (and possibly other ingredients, such as methoprene) in proportions that will result in enhanced sterile male performance in the field is still not fully understood, and consequently such approaches have not been implemented by many operational programmes. The Moscamed programme in Mexico uses the Mubarqui adult diet for C. capitata, which contains proteins from diverse plant seeds (Gómez et al. 2013), and the Moscafrut programme in Mexico releases sterile A. ludens and A. obliqua flies fed with a 24:1 sugar:yeast adult diet (Pereira et al. 2021).

Hormonal treatment. Research on several *Anastrepha* species showed that juvenile hormone regulates sexual maturity and sexual signalling in males (see Pereira et al. 2021 for a recent review). Application of juvenile hormone analogues, such as methoprene, accelerates reproductive development and sexual signalling in *Anastrepha* and *Bactrocera* species (Adnan et al. 2018, Teal et al. 2013). Methoprene has been shown to further improve male sexual performance in *A. ludens* and *A. fraterculus* (Pereira et al. 2010, Bachmann et al. 2017). For some species, the effect of methoprene on sterile males was only achieved when hormone treatment was coupled with a protein-enriched pre-release diet (Teal et al. 2013). This advantage is particularly important for SIT application against species that have long pre-copulatory periods (like *Anastrepha, Bactrocera,* and *Z. cucurbitae*). Considerable progress has been made in developing delivery systems to treat large numbers of flies with methoprene in operational programmes, particularly providing this analogue as part of the pre-release diet (Gomez-Simuta et al. 2016; Adnan et al. 2020).

Semiochemicals. Males of most *Anastrepha*, Bactrocera, Ceratitis and Zeugodacus species are attracted to natural compounds known as semiochemicals (Segura et al. 2018). Some species sequester these chemicals for use in pheromone synthesis; like methyl eugenol (ME) by some *Bactrocera* species (Tan and Nishida 1996). Ingestion of ME by males increased their mating success. In *C. capitata*, semiochemicals released by ginger root oil (GRO) or citrus oils increase the mating competitiveness of males (Papadopoulos et al. 2001; Shelly 2001). In *Anastrepha*, exposure to fruit volatiles increased the mating success in some species (Vera et al. 2013, Morató et al. 2015). Research carried out in this area has helped to understand these phenomena, to extend them to other species, and to transfer and validate them under the largescale conditions of action SIT programmes (Pereira et al. 2021).

The semiochemicals that can improve sterile males mating success are the following (Segura et al. 2018, Pareira et al 2021):

- Methyl eugenol in several *Bactrocera* species, including *B. correcta*, *B. dorsalis,* and *B. zonata* males (Tan and Nishida 1996, Quilici et al. 2004, Shelly et al. 2005, Obra and Resilva 2013).

- Cuelure in *B. tryoni* and *Z. cucurbitae* males (Weldon et al. 2008; Shelly 2019).

- Raspberry ketone and/or zingerone in *B. tryoni* and *Z. cucurbitae* males (Khoo and Tan 2000; Akter et al. 2017b; Akter and Taylor 2018; Shelly 2019).

- a-copaene in *C. capitata* (Shelly et al. 2001).

Other sources of semiochemicals that have shown to enhance male mating competitiveness include:

- Ginger root oil in *C. capitata* and *C. quilicii* (Shelly 2001; Shelly et al. 2007a; Quilici et al. 2013).

- Manuka oil in *C. capitata* (Shelly et al. 2008c).

- Citrus oils in *C. capitata* and *C. quilicii*, and *A. fraterculus* (Shelly 2001; Shelly et al. 2007; Quilici et al. 2013; Ruiz et al. 2021).

- Citrus fruit in *A. ludens* (Morató et al. 2015).

- Guava fruit and guava essential oil volatiles in *A. fraterculus* (Vera et al. 2013; Bachmann et al. 2015; Belliard et al. 2021).

Methodologies for exposing large numbers of C. capitata males through GRO or citrus-oil aromatherapy on a large scale in adult-holding rooms at fly emergence and release facilities have been developed (Shelly et al. 2007c, 2008a). They are now applied in a cost-effective manner in on-going SIT programmes in Australia, Croatia, Guatemala, Israel, Mexico, Spain, and the USA. In the case of ME, Haq et al. (2014b, 2015, 2018) demonstrated that ME application by aromatherapy also enhanced the mating success of males of *B. carambolae* and *B. dorsalis*. This method appears to have merit for adoption but needs to be evaluated at larger scales.

Gaps Identified

- Effects of manipulating the holding environmental conditions and duration of this phase, either separately or in combination with nutritional, hormonal, and semiochemical treatments, on subsequent male quality in the field.
- Release methods, while operationally convenient, are not always optimal in terms of sterile male performance. For example, the effect of chilling (to immobilize) collected flies for aerial release

can at least temporarily affect sterile male flight ability and mating competitiveness, including pheromone quantity or quality

- The effects and interactions of the different processes, treatments, and systems need to be further assessed and refined, tailoring them to the biology of each target fruit fly species. Particularly, interaction between methoprene and semiochemicals has not been fully addressed.
- Cost effective and practical alternatives to provide protein or nitrogenous compounds to emerging sterile males.
- Efficient methods to deliver methoprene have not been established in many species, particularly in the genus *Bactrocera*.
- There is a lack of knowledge on the chemical basis of the response of males to semiochemicals release by complex sources (e.g., fruits, essential oils).
- Slow adoption of supplements by SIT operational programmes due to practical reasons.
- Nutritional needs of males requiered to expresse their maximun reproductive potential while balancing important trade-offs between sexual performance and survival.

4.3 FIELD OPERATIONS

4.3.1 Improved Trapping Systems (Traps and Attractants)

Participants: Bishwo Mainali, David Nestel, Julio Rojas*/Pablo Liedo, Martha Martinez/Ignacio Pla, Preeaduth Sookar* Polychronis Rempoulakis

Background Situation Analysis

Accurate methods for fruit fly population surveys are a prerequisite for effective decision-making in area-wide control programmes aimed at pest suppression, as well as those attempting to establish fruit fly free or low prevalence areas. The specific trapping system to be used should depend on the objective of the pest control programme, economic and technical feasibility, the target species of fruit fly and the phytosanitary condition of the delimited areas, which can be either an infested area, an area of low pest prevalence, or a pest free area.

Pheromones or parapheromones that are effective, selective and male-specific are available for the main species of *Bactrocera* and *Zeugodacus* of economic significance as well as for the Mediterranean fruit fly (*Ceratitis capitata*). However, one constraint in the case of the *Bactrocera* and *Zeugodacus* species is the lack of an effective female attractant which affects population monitoring and fruit fly control programme evaluation specially when the sterile insect technique is applied.

In the case of trapping systems for fruit flies of the genus *Anastrepha* the situation is the contrary, where attractants are limited to female biased food-based attractants which are not so efficient and selective. There is an urgent need to develop more powerful male or female specific attractants for these fruit fly species in order to improve population monitoring and the overall programme management.

Large-scale fruit fly control programmes spend millions of dollars in maintaining extensive trapping networks. The possibility of developing smart traps has been seen in the past few years as having great potential to reduce costs of operating trapping networks, and to be more.

Smart traps which use sticky traps or pheromone traps combined with cameras or other type of sensors have been developed for commercial use (Schellhorn and Jones, 2021). Low cost 'Smart Traps' may

be deployed in big orchards or cucurbit plantations to improve the trapping and detection system so that farmers can apply fruit fly control measures on time.

Smart traps using several types of sensors exists, and are in the direction of being commercialized, or are already commercial. Most of these traps attract fruit flies using specific attractants. Trapped fruit flies are counted using all sort of sensors and systems, such as behavioural fingerprints, or the interruption of laser beans. However, in any of these cases, fruit flies being captured are visually identified, thus other insect entering traps can be misclassified and counted.

Current Knowledge

The most widely used attractants are pheromones or parapheromones that are male-specific. The parapheromone trimedlure (TML) captures species of the genus *Ceratitis* (including *C. capitata* and *C. rosa*). Alternatives to TML include Capilure which is a type of TML with extenders to slow down volatilization and increase the service interval of the trap. The parapheromone methyl eugenol (ME) captures a large number of species of the genus *Bactrocera* (including *B. dorsalis, B. zonata*, *B. carambolae*, *B. correcta* and *B. musae)*. The parapheromone cuelure (CUE) captures a large number of other *Bactrocera* species, including *B. tryoni* as well as *Zeugodacus cucurbitae*. Female-biased attractants (natural, synthetic, liquid or dry) that are commonly used are based on food or host odours. Several food-based synthetic attractants have been developed mainly for *C. capitata* using ammonia and its derivatives. This may reduce the number of non-target insects captured.

For fruit flies belonging to the genus *Anastrepha*, food-based attractants are available such as hydrolyzed proteins or torula yeast. The enzymatically hydrolyzed protein of animal origin (Ceratrap) is an attractant that has been shown to have a greater power of attraction than conventional proteins of plant origin. About 25 years ago, a synthetic attractant made with ammonium acetate, putrescine (2 CL) was developed for use against *Anastrepha* species. A new formulation of this lure (vial-lure) is now commercially available. Research has been conducted with pheromone compounds and host fruit volatiles (kairomones), but these have not become commercially available attractants yet. Parapheromones such as methyl eugenol, cuelure or trimedlure do not exist for *Anastrepha* and lures with such attractancy power are still highly desirable.

Smart traps are equipped with special high-resolution micro cameras or with optic devices capable of generating digital images. The images are automatically transmitted to the cloud and to a central laboratory where the flies are identified. When there is uncertainty on the species of fruit fly that has been caught the specimen can be collected in the field and brought into the laboratory for identification by a taxonomist under a microscope. The traps are wireless and the devices inside the traps can be operated using batteries or with individual solar cells or a generator powered with solar energy. Other similar traps are based on sound and the recognition of the fruit fly species by wing beat.

These traps represent an important innovation as it could substantially reduce the cost of operating extensive trapping networks. This would be especially important for surveillance networks placed at high-risk points of entry such as airports, seaports and border crossings which are normally checked and serviced on-site every two weeks. Traps can also be deployed in production systems where low prevalence of the fruit fly pest species exists.

The cost savings would come from reducing the number of human-hours dedicated to checking the traps for fruit fly captures every week or every two weeks. In addition, the cost savings in fuel when trappers must drive sometimes long distances to reach the traps.

Another significant advantage is that the smart traps are early warning systems. Fly catch can be transmitted in real time with proper connectivity as the transmission of images is on a continues basis supporting management decisions. This is especially important for surveillance networks aimed at early detection of invasive fruit fly species. When an incursion of a fly occurs an immediate emergency, response is triggered to characterize the profile of the pest incursion and in the case of an outbreak, implement eradication actions. Recently, image analysis algorithms are being developed and integrated into the analytical systems to classify fruit fly species, and separate between them (I.e., *Ceratitis capitata, Bactrocera dorsalis* and *B. zonata*). This novel system is expected to provide alerts and numerical data that can be incorporated into geographic and population modelling.

A number of prototypes of fruit fly automated traps have been developed, tested and already in use in some countries such as Australia and the USA (Schellhorn and Jones, 2021). This includes modified versions of the McPhail trap, Lynfield trap, and Jackson trap.

Such state-of-the-art innovations will increase the feasibility of area-wide integrated pest management programmes as well as optimize the management of surveillance networks against invasive fruit fly quarantine species.

Gaps Identified

- In general, there is a need for more effective and selective attractants including parapheromones for some species of *Anastrepha, Bactrocera, Ceratitis and Dacus* fruit flies.
- There is a need to develop more selective and effective female biased traps against economic species of the genus *Bactrocera and Dacus.*
- Current smart-traps prototypes based on images require to be improved (energy-wise) and upscaled to reduce productions costs and make surveillance programs more efficient.
- There is a need to develop models and strategies to improve the geographic trap deployment systems in low-prevalence areas and in free areas to increase interception probabilities.
- There is the need to adopt decision support tools to improve management of trapping networks.
- Standard FAO/IAEA protocol for trap evaluation.

Note - Participants may follow the standard protocol for trap evaluation available in Annex 5.

A standardized research protocol for trap evaluation was developed by the FAO/IAEA and a group of consultants. The protocol was used to evaluate traps and attractants as well as bait stations in the CRP "Standardization of medfly trapping for use in sterile insect's technique programmes" conducted from 1986 to 1992, in the CRP "Development of Improved Attractants and Their Integration into Fruit Fly Management Programmes" conducted from 1994 to 1998 and in the CRP "Development of improved Attractants and Their Integration into Fruit Fly SIT Management Programmes" conducted from 2000 to 2005.

As a result, the female biased attractant three and two component lures (Biolure) were developed and validated against a range of economic fruit fly species as well as fruit fly traps including the Multilure trap (McPhail type) and the Tehpritrap. These attractants and traps are extensively being used by FAO and IAEA Member States.

CRP participants interested in evaluating trapping systems may choose to follow the IAEA standard protocol for trap evaluations available in Annex 5 of this report. Participants should refer to this protocol

in the 5 year and 18 months plan indicating the fruit fly species of interest, the traps and/or attractants that will be evaluated and the timelines.

4.3.2 Decision support tool for optimization of surveillance networks

Participants: Nick Manoukis*, Polychronis Rempoulakis, Katharina Merkel, Preeaduth Sookar, David Nestel, Mariel Vanin

Background Situation Analysis

As mentioned above, trap networks are important components of most action programmes against Tephritid fruit flies globally. They fill important roles from surveillance to delimitation to programme effectiveness estimation. Despite their criticality, it remains difficult to quantitatively assess the sensitivity and effectiveness of trap networks, limiting the extent to which they can be optimized.

A specific needs of SIT programs is to estimate the size of wild populations to allow an adequate overflooding ratio. The capability to estimate population sizes of wild flies might be attainable using computer simulations and models if biological parameters are estimated based on field experiments. Important parameters include trap attraction (relationship between distance and probability of capture), movement, and proportion responsive insects. These can be evaluated via Mark-release-recapture experiments as well as behavioral assays.

Current Knowledge

In the last few years, a computational simulation model, TrapGrid, has been developed that can help to address questions of how to improve trap network design and operation. TrapGrid is a spatially-explicit that simulates insect movement and capture in a network of attractant-baited traps (Manoukis et al 2014). To date a key parameter, trap attraction, has been estimated in the field via Mark-Release-Recapture (MRR) experiments for *B. dorsalis*/ Methyl Eugenol, *C. capitata* /Trimedlure, and *Z. cucurbitae*/ Cuelure (Manoukis et al 2015; Manoukis and Gayle 2016). Movement parameters for simple diffusion are available from the literature for some species, but for the more realistic movement model (Random Correlated Walk, RCW), we are not aware of any field estimates for tephritids.

Implementation of existing models such as TrapGrid into accessible tools (such as an Excel-based spreadsheet or a web app) is another important development that would enhance uptake of these powerful models. Benefits to programmes would include better targeting of available sterile insect and trapping resources, quantification of programme impact, and enhanced adaptability to changes in pest populations.

Gaps

The critical attraction parameter, lambda, has only been estimated for a few species with male lures. The parameter has not been assessed for food-based lures, widely relied on for many tephritids especially in the genus *Anastrepha*.

Movement parameters are knows from the literature for simple diffusion, but new estimates could be helpful for additional species. Furthermore, RCW parameters are generally unavailable, precluding application of this more realistic movement model.

The geographic component in the deployment of monitoring and surveillance networks is poorly developed and is high in labor and transportation costs. Better systems are required to rank the landscape probability of bearing fruit flies, and the probability of trapping flies, if existent. This is highly relevant for surveillance of alien fruit flies, and for areas of low prevalence and fruit fly free.

The combination of the TrapGrid and a landscape ranking system, may provide a good platform to reduce cost, improve trapping probabilities, and reduce risk. Moreover, the addition of smart traps strategically deployed will undoubtedly make the surveillance systems more efficient and drastically reduce costs.

4.3.3 Improve Fruit Fly Suppression Through Validation and Harmonization of Bait Stations

Participants: Karim Nebie, Pedro Rendon, Preeaduth Sookar*, Katharina Merkel, Mariel Vanin

Background Situation Analysis

To reduce the populations of fruit fly pests, mixtures of protein or food attractant with chemical products have traditionally been used as foliar sprays. Typically, organophosphate products are used as insecticides blended with these baits. Currently, there are complementary alternatives in the form of bait stations (using spinosad, as an active ingredient) that have demonstrated to reduce the populations of several species of fruit flies in addition to being compatible with organic production (Rendon et al. 2000). It has also been documented these do not have the negative environmental consequences of some insecticides including the avoidance of damage to pollinators, invaluable for agriculture.

To solve the recurring problem of not being able to spray on backyard crops, particularly in rural populated areas or major cities, tourist areas, national parks, protected areas and abandoned crops, bait stations have been developed. Bait stations described here use the same food attractants used in the trapping system for C. capitata and *A. ludens* which mainly attract female flies (active agent of reproduction) of these species towards the surface of the unit, which is impregnated with the same killing agent/active ingredient (spinosad) used in aerial bait sprays. The design of these bait stations allows their use in combination with other control methods (i.e. biological control), they do not represent a risk to pollinators are biodegradable. It has been determined that the units could last for more than twelve weeks in the field, which makes their use very practical and economical, also solving the existing problem of continuous re-infestations generating within untreated areas due to access or others types of restrictions.

Generally, bait stations target both male and female fruit flies. Some bait stations target females. Previous studies have shown that bait stations can be effectively used to control *A. obliqua* and *A. ludens* in mango orchards in Chiapas, Mexico (Flores et al. 2017). Jemâa et al. (2010) reported that mass trapping using a female-targeted lure (Tri-pack®, Kenogard SA, Barcelona, Spain), successfully controlled of Ceratitis capitata. Studies on bait sprays and bait stations as a complementary tool has given effective control of *Anastrepha* flies (Díaz-Fleischer et al. 2017) and C. capitata female populations in citrus orchards (Leza et al. 2008). There is a pressing need to assess further the integrated

use of bait sprays and bait stations for areawide control of the *Bactrocera* spp. Research should be geared towards the development of an ideal bait station which has low cost and low environmental impact and is easy to use, selective (target female fruit flies), long lasting, safe, and easy to install.

Current knowledge

Like ground bait sprays, bait stations is not a stand-alone control method for effective fruit fly suppression but should be integrated with a series of other control methods. Bait stations should be an effective complementary tool either for area-wide suppression, eradication and exclusion scenarios as for use in fruit and vegetable commercial areas aimed at producing commodities for export and local markets.

The timing of deployment of bait stations in the field and the layout of the bait station deployment should be based on pest and host ecology data. These data should include information on biotic factors such as overwintering/aestivation of populations, availability of host/shelter trees, breeding sites, fruit host phenology, and also on abiotic factors such as temperature, humidity, rain, winds, etc. In commercial crops bait stations should be deployed in the field early to prevent population build-up. A homogenous layout of bait stations would be the most common application in areas with uniform host distribution. However, deployment in hot-spots or random layouts could be used for highly patchy or unknown pest and host distributions. Another option is the use of a gradient of bait stations with higher densities in the periphery to protect the target area, as it is currently recommended when applying ground baits sprays in commercial orchards or for protecting places of production surrounded by an area of low pest prevalence as a buffer.

Densities of bait stations should be determined based on a number of factors including pest density, occurring pest physiological stage, efficiency of the attractant and killing agent, phenology, host density and objective of the programme. For commercial areas value and susceptibility of the host can also be taken into consideration. In this latter case, there is plenty of information pointing out that in a single host species there can be some varieties that are more susceptible than others so that density of bait stations may vary in each case.

Gaps Identified

Develop new, more powerful and long-lasting attractants that can increase bait station effectiveness.

Development of effective and environmentally-friendly killing agents (e.g. entomopthogens), and integration of visual and olfactory cues.

Detailed knowledge of fruit fly population ecology is essential for timing the deployment of BS in the field as well as for assessing the spatial distribution of BS. If fruit fly spatial distribution within a commercial orchard or in marginal host areas is known, bait stations may be aggregated to overlap with the fruit fly population. Knowledge of the dispersion behavior of fruit flies from areas surrounding the orchard into the orchard, may be used to deploy bait stations around the orchard's periphery before the flies move into the orchard to reduce or eliminate immigrating flies (Alemany et al. 2004).

Economic feasibility assessments of the use of BS are required to support decision making between the use of this technology and other alternate technologies aimed at fruit fly population suppression. Non-target effects to demonstrate the environmental benefits of BS should be part of the variables to quantify in the assessment.

Conducting side-by-side comparisons of the various bait station types that have been developed in recent years to determine actual effectiveness against multiple fruit fly species in various geographical areas and using standardized methodologies. BS evaluation must ultimately be based on fruit infestation levels.

- **Standard Protocols for Bait Station Evaluation**

The Joint FAO/IAEA Division in partnership with many collaborators and stakeholders has developed standardized methodologies for bait station research (FAO/IAEA 2007). These methodologies have been used in Argentina and Spain to evaluate bait stations and mass trapping. The Moscamed Programme in Guatemala (USDA-APHIS) has also developed standard methodologies for bait station evaluation that have been used in Guatemala and Texas, USA. Pedro Rendon and Walther Enkerlin to provide guidelines for bait station evaluation.

Field evaluation of bait stations should include:

- Comparison of effectiveness with the conventional international standard. Particularly with the ground bait sprays internationally used. These can be the standard combination of malathion/hydrolysed protein and/or GIF-120 spinosad baits;
- Evaluation at a sufficiently large scale to determine cost-effectiveness;
- Use of an area-wide approach, including buffer zones, to minimize the distorting effects of immigrating flies that are attracted to the core area from the surrounding areas;
- Population sampling combining traps for adults and fruit sampling to determine larval presence in fruit. Adult trapping allows for self-correction (results can be analysed during the test), but ideally the final evaluation should be based on percentage fruit infestation just before harvest.

V. Logical Framework

New CRP Proposal On "Improving Rearing, Handling, And Field Components For Fruit Fly SIT Application"

LFM-Logical Framework Matrix Input:

Overall Objectives:

The main objective of this CRP is to optimize and harmonize through applied research the use of SIT and related technologies for management of plant pests.

Specific Objectives:

Optimize sterile fly production by improving mass rearing technologies and improved genetic sexing strains (GSS).

Improve area-wide SIT application by enhancing sterile fly quality and by introducing more cost-effective technologies and decision-making tools for sterile fly release.

Optimize fruit fly surveillance and control by introducing improved trapping systems and decision-making tools for management of trapping networks, as well as bait stations.

Outcomes:

1. Improved sterile insect technique through more efficient mass rearing methods including the utilization of improved GSS.
2. Improved sterile insect technique through more efficient sterile fly pre-release handling and through decision support tools for optimization of sterile fly release.
3. Improved fruit fly surveillance and control through more efficient trapping systems and through decision support tools for management of trapping networks and effective control methods.

Outputs:

Improved area-wide SIT through maintaining high genetic diversity by introducing wild gens into GSS breeding colonies

1.1 Improved area-wide SIT through maintaining high genetic diversity by introducing wild gens into GSS breeding colonies.
1.2 Protocol for maintaining GSS breeding colonies with high genetic diversity available and adopted by mass rearing facilities.
1.3 Quality of sterile insects improved through maintaining high genetic diversity in the GSS breeding colony.
1.4 Mass production of fruit flies enhanced though improved gel diets.

2.1 Quality of sterile insects improved by providing food supplement to adults prior to field releases.
2.2 Decision models to optimize sterile fly aerial release adapted to a range of fruit fly species.
2.3 Cost-effectiveness of SIT improved through decision models to optimize sterile fly aerial release.

3.1 Fruit fly monitoring and detection improved through more efficient traps and attractants.
3.2 Surveillance networks optimized, and early detection of quarantine species enhanced using decision-making models for trap management.
3.3 Fruit fly control improved through more efficient population suppression tools.

4. Results published in a peer reviewed journal.

Activities:

1. Announce project amongst established entomologists working in fruit fly area-wide SIT operational programmes.
2. Organize first RCM to refine the logical framework and plan the overall activities of the CRP (4Q 2021).
3. Provide necessary research protocols to contract holders.
4. Supply specific materials for research to contract holders.
5. Conduct applied research and development.
6. Organize second RCM to analyse progress in delivering research outputs and plan the next phase of the project (1Q 2023).
7. Supply specific materials for research to contract holders.
8. Conduct applied research and development.
9. Review the CRP after its third year.
10. Organize third RCM to analyse progress in delivering the research outputs and plan the final phase of the project. (3Q 2024).
11. Supply specific materials for research to contract holders.
12. Conduct applied research and development.
13. Organize final RCM to assess the success of the CRP in reaching its objectives and review the final publication. (1Q, 2025).
14. Evaluate the CRP and submit evaluation report.
15. Publish the results of the CRP in a special issue of a peer reviewed journal.

Logical Framework (table):

Elements	***Objective Verifiable Indicators***	***Means of Verification***	***Important Assumptions*** ***(Mainly for CSI's)***
Overall Objective The main objective of this CRP is to optimize the use of SIT and related technologies for management of fruit fly pests	N/A	N/A	The use of SIT for fruit fly management is expanding in Member States. Increasing cost-effectiveness of SIT technology is critical for adoption of the technology by more Member States. For research aimed at optimizing SIT and related technologies, mass- rearing and field operation programmes should be available in Member States.

Specific Objectives			
1. Optimize sterile fly production by improving mass-rearing technologies and use of improved GSS strains	Improved production volumes and insect quality	Reports, protocols and published papers.	Managerial support and availability of expertise and resources required to conduct large-scale applied research in mass-rearing and irradiation.
2. Improve area-wide SIT application by enhancing sterile fly quality and by introducing more cost-effective technologies and decision-making tools for sterile fly release.	Enhanced sterile fly quality indices and sterile fly release parameters	Reports, decision making models and published papers.	Managerial support and availability of expertise and resources required to conduct large-scale applied research to improve sterile male performance and aerial release.
3. Optimize fruit fly surveillance and control by introducing improved trapping systems and decision-making tools for management of trapping networks and bait stations.	Sensitivity and management of trapping networks improved. Increased effectiveness in population suppression.	Reports, decision making models and published papers.	(**Same as above**) Managerial support and availability of expertise and resources required to conduct large-scale field experiments to improve surveillance systems and population suppression.

Outcomes (Results) 5. Improved sterile insect technique through more efficient mass-rearing methods and improved GSS strains.	Increased yields and improve sterile fly quality indices. Production protocols available.	Technical reports, published papers. Protocols adopted.	Improved technologies, improved GSS and tools adopted by MS.
6. Improved sterile insect technique through more efficient sterile fly pre-release handling and through decision support tools for optimization of sterile fly release.	Enhanced sterile fly quality indices and sterile fly release parameters Decision making tool for aerial release of sterile insects available.	Technical reports, published papers. Sterile fly density model adopted.	(**same as above**) Improved technologies and tools adopted by MS.
7. Improved fruit fly surveillance and control through more efficient trapping systems and through decision support tools for management of trapping networks and effective control methods.	Sensitivity and management of trapping networks improved. Increased effectiveness in population suppression.	Technical reports, published papers. Trapping models adopted.	(**same as above**) Improved technologies and tools adopted by MS.

Outputs (products)			
1.1. Improved area-wide SIT through maintaining high genetic diversity by introducing wild gens into GSS breeding colonies	New GSS introduced to breeding colonies in at least two programmes	Reports and / or published papers	Genetic model for maintaining genetic diversity available for evaluation
1.2 Protocol for maintaining GSS breeding colonies with high genetic diversity available and adopted by mass rearing facilities	At least two protocols for maintaining GSS colonies with high genetic diversity adopted	Reports and / or published protocols	Necessary means available for adopting the new genetic GSS
1.3 Quality of sterile insects improved through maintaining high genetic diversity in the GSS breeding colony	Improved sterile fly quality parameters	Reports and / or published papers	Methods for QC assessment available
1.4 Mass production of fruit flies enhanced though improved gel diets	Increase sterile fly production yields	Reports and / or published manual	Diet ingredients commercially available
2.1 Quality of sterile insects improved by providing food supplement to adults prior to field releases	Improved sterile fly quality parameters including mating performance and fliers	Reports and / or published papers	Methods and resources for QC assessment available
2.2. Decision models to optimize sterile fly aerial release adapted to a range of fruit fly species	Improved sterile fly release parameters including percent fly distribution and abundance (FTDs)	Reports and / or published manual	Methods and resources for QC assessment available
2.3 Cost-effectiveness of SIT improved through decision models to optimize sterile fly aerial release	Decision model adopted and in use	Reports and / or published papers	Managerial support to adopt the model

3.1 Fruit fly monitoring and detection improved through more efficient traps and attractants	Increased sensitivity of traps measured through FTD index.	Reports and / or published papers	Methods and resources to measure trap efficiency available
3.2 Surveillance networks optimized, and early detection of quarantine species enhanced using decision-making models for trap management	Decision model adopted and in use	Reports and / or published papers	Managerial support to adopt the model
3.3 Fruit fly control improved through more efficient population suppression tools	Increased efficiency for population suppression measured through FTD and fruit infestation index.	Reports and / or published papers	Methods and resources to measure FTDs and fruit infestation available
8. Results published in a peer reviewed journal.	Papers drafted and submitted.	Journal special issue with published scientific papers.	Data and articles for publication available

Activities			
1. Announce project amongst established entomologists working in fruit fly area-wide SIT operational programmes	Proposals evaluated and 11 Research Contracts, 8 Research Agreements	Signed contract and agreements	Suitable proposals submitted, funding available and approval of Contract and Agreements by CCRA-NA committee.
2. Organize first RCM to refine the logical framework and plan the overall activities of the CRP (4Q 2021)	1st RCM held virtually 1–5 November 2021	Participants' activities and logical framework revised. Reports and protocols	Contracts and Agreements signed by counterpart organizations.
3. Prepare necessary research protocols to contract holders	Research protocols available	Procurement orders available	Research protocol will be implemented by qualified scientists.
4. Supply specific materials for research to contract holders	Specifications and request for procurement	Scientific papers and reports from the participants	Support to enter the procurement items into the MS.
5. Conduct applied research and development	New knowledge created on mass rearing, dosimetry, sterile fly release and population suppression	Participants and RCM Progress Reports.	Methods and resources available.

6. Organize second RCM to analyse progress in delivering research outputs and plan the next phase of the project (2Q 2023).	2nd RCM will be held 2Q 2023 in Vienna, Austria	Procurement orders available	Progress satisfactory.
7. Supply specific materials for research to contract holders	Specifications and request for procurement	Scientific papers and reports from the participant Report	Support to enter the procurement items into the MS.
8. Conduct applied research and development	New knowledge created on mass rearing, sterile fly release and population suppression	Participants and RCM Progress Reports.	Methods and resources available.
9. Review the CRP after its third year (Midterm review)	Satisfactory progress of research agreements and technical contract	Participants and RCM Progress Reports.	Contracts and Agreements properly managed by counterpart organizations. Methods and resources available.
10. Organize third RCM to analyse progress in delivering the research outputs and plan the final phase of the project. (4Q 2024)	3rd RCM to be held 4Q 2024.	Procurement orders available	Progress satisfactory and mid-CRP evaluation approved by CCRA-NA committee.
11. Supply specific materials for research to contract holders	Specifications and request for procurement	Scientific papers and reports from the participant	Support to enter the procurement items into the MS.
12. Conduct applied research and development	New knowledge created on mass rearing, dosimetry, sterile fly release	Participants and RCM Progress Reports.	Methods and resources available.

	and population suppression		
13. Organize final RCM to assess the success of the CRP in reaching its objectives and review the final publication. (2Q, 2026)	4th RCM to be held 2Q 2026.	Participants and RCM Final Reports	Final reports are submitted to the Agency.
14. Evaluate the CRP and submit evaluation report.	Satisfactory completion of research agreements and technical contract	Report	Contracts and Agreements properly managed by counterpart organizations. Methods and resources available.
15. Publish the results of the CRP in a special issue of a peer reviewed journal.	At least 20 publications accepted.	Scientific publications.	Consensus can be found on appropriate peer review journal and acceptance by journal obtained.

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ANNEX 1

LIST OF PARTICIPANTS

D4.10.29-CR-1

First Research Coordination Meeting on Improving Rearing, Handling, and Field Components for Fruit Fly SIT Application

Vienna, Austria (virtual)

1 to 5 November 2021

List of Participants

(as of 2021-10-28)

1	**Argentina**	Mr Diego Fernando SEGURA Instituto Nacional de Tecnología Agropecuaria Rivadavia 1439 1033AAE BUENOS AIRES ARGENTINA Email: segura.diego@inta.gob.ar
2	**Argentina**	Ms Mariel VANIN Instituto de Sanidad y Calidad Agropecuaria Mendoza (ISCAMEN) Av. Boulogne Sur Mer P.O. Box 3050 5500 MENDOZA ARGENTINA Tel: +5402614258741 Email: vanin.mariel@gmail.com
3	**Australia**	Mr Bishwo MAINALI Department of Biological Sciences Room G-35 205b Culloden Road Macquarie University NSW 2109 SYDNEY AUSTRALIA Email: Bishwo.Mainali@mq.edu.au
4	**Australia**	Ms Katharina MERKEL South Australian Research and Development Institute Government of South Australia | E106 Main Waite Building GPO Box 397 ADELAIDE, SA 5001 Postal Code: 5064 AUSTRALIA Email: Katharina.Merkel@sa.gov.au

5	**Australia**	Mr Polychronis REMPOULAKIS University of Newcastle Ourimbah Campus, North Loop Road, Ourimbah NSW 2258, Locked Bag 26 GOSFORD NSW AUSTRALIA Tel: +972 (0)506746234 Email: polychronis.rempoulakis@dpi.nsw.gov.au
6	**Brazil**	Mr Valter ARTHUR Centro de Energia Nuclear na Agricultura; Universidade de Sao Paulo (USP) Avenida Centenario, 303; Piracicaba 13400-970 SÃO PAULO BRAZIL Email: arthur@cena.usp.br
7	**Brazil**	Mr Dori NAVA Empresa Brasileira de Pesquisa Agropecuária Embrapa Clima Temperado Br 392 km 78, Caixa Postal 403 PELOTAS BRAZIL Email: dori.edson-nava@embrapa.br
8	**Burkina Faso**	Mr Karim NEBIE Institut de l'Environnement et de Recherches Agricoles 01 BP 910 BOBO-DIOULASSO BURKINA FASO Email: nebkar87@gmail.com
9	**Guatemala**	Mr Cristian Rivelino MORALES ALONZO Medfly Program 16 calle 3-38 Zone 10 GUATEMALA Email: cristian.morales@medfly.org.gt
10	**Israel**	Mr David NESTEL Department of Entomology; Volcani Centre; Agricultural Research Organization; Ministry of Agriculture and Rural Development P.O. Box 6 50250 BET DAGAN ISRAEL Email: nestel@agri.gov.il

11	**Mauritius**	Mr Preeaduth SOOKAR Entomology Division; Agricultural Services; Ministry of Agro-Industry and Food Security St. Jean Ro 00 REDUIT MAURITIUS Tel: 00230 4664983 Email: psookar@govmu.org
12	**Mexico**	Mr Jose Mauricio ESTEBAN SANTIAGO National Program Fruit Flies SAGARPA-SENASICA Direccion General de Sanidad Vegetal Boulevard Adolfo Ruiz Cortines 5010 04530 CIUDAD DE MEXICO MEXICO Email: jose.esteban@senasica.gob.mx
13	**Mexico**	Mr Carlos PASCACIO-VILLAFAN Instituto de Ecologia, A.C. (INECOL) Carretera antigua a Coatepec 351 El Haya 91070 XALAPA VERACRUZ MEXICO Email: carlos.pascacio@inecol.mx
14	**Mexico**	Mr Julio Cesar ROJAS LEON El Colegio de la Frontera Sur (ECOSUR) Carretera Antiguo Aeropuerto Km 2.5 30700 TAPACHULA CHIAPAS MEXICO Email: jrojas@ecosur.mx
15	**South Africa**	Mr Christopher WELDON Flies of Economic Significance Research Group Room 2-8, Department of Zoology and Entomology University of Pretoria, Private Bag X20 HATFIELD SOUTH AFRICA Tel:+27 (12)420 3234 Email: cwweldon@zoology.up.ac.za

16	**Spain**	Ms Marta MARTÍNEZ GONZALVO Empresa de Transformación Agraria S.A., S.M.E., M.P. (TRAGSA) Avda. Industria, 28 - Pol. Ind. L'Andana 46980 PATERNA SPAIN Email: mmarti79@tragsa.es
17	**United States of America**	Mr David HAYMER School of Medicine University of Hawaii 651 Ilalo St. 96813 HONOLULU HI UNITED STATES OF AMERICA Email: dhaymer@hawaii.edu
18	**United States of America**	Mr Nicholas C MANOUKIS PBARC; Agricultural Research Service (ARS); US Department of Agriculture (USDA) 64 Nowelo St HILO, HI UNITED STATES OF AMERICA Email: nicholas.manoukis@usda.gov
19	**Viet Nam**	Ms Thi Kim Lien HA Plant Protection Research Institute Duc Thang Tu Liem 000084 HANOI VIET NAM Tel: +84 0975668679 Email: haliennhomncr@gmail.com

ANNEX 2

MEETING AGENDA

First Research Coordination Meeting (RCM)

"D41029: Improving SIT Fruit Fly Field Programmes"

Vienna, Austria, 1–5 November 2021

DAY	ACTIVITY	TIME	PRESENTER
1 November Day 1 (9:00 pm - 12:40 am Vienna time)	Welcome and objectives Agenda Research Coordination Meeting (RCM) (goals and methodology) CRP Perspectives **Presentations of Research Proposals** • Genetic model for refreshment of breeding colonies • Improving SIT and field components - GSS strains and gel diets / Improved trapping • Improvement of the rearing and the genetic background of the Medfly genetic sexing strain • Improving the field performance of *Anastrepha fraterculus* sterile males through specific refreshing protocols and pre-release treatments • Development and evaluation of genetic sexing strains for *Anastrepha fraterculus* to enable sterile male-only releases in Brazil **Break** • Studies in Biofactories on Nutritional Larval Diets and Development and Maintenance of Genetic Sexing Strains of Two Species of Fruit Flies of Economic Importance • Use of a gelling/texturing agent to replace agar in artificial diet for *Anastrepha fraterculus* larvae, aiming at the sterile insect technique and biological control	5 min 5 min 10 min 10 min 20min 20min 20 min 20min 20min 10 min 20min 20min	Rui Cardoso/Walther Enkerlin All Walther Enkerlin Walther Enkerlin Carlos Caceres Mariel Vanin (Argentina) Salvador Meza/Jose Santiago (Mexico) Diego Segura (Argentina) Valter Arthur/ Thiago Mastrangelo (Brazil) Cristian Morales/Edwin Ramirez (Guatemala) Dori Nava (Brazil)

	• Development and Optimization of Gel Diet Rearing Systems for Improving the Sterile Insect Technique Against *Anastrepha ludens* and Ceratitis capitata • Improvements for rearing and performance of sterile fruit flies through manipulation of dietary lipids	20min 20min	Carlos Pascacio-Villafán Mexico) Cristopher Weldon (South Africa)
2 November Day 2 (9:00 pm – 12:50 am Vienna time)	**Presentations of Research Proposals** • Enhancing fruit fly sterile insect technique through improved and cost-effective gel larval diet, pre-release handling, and monitoring • Optimize fruit fly production and rear out systems, improving fruit fly management practices, enhance fruit surveillance and control by introducing improved trapping systems and decision-making tools for management of trapping networks • Improving rearing and control techniques with the integrated use of SIT for *B. dorsalis*, *B. zonata* and *Z. cucurbitae* • Influence of Dietary Protein on Performance of Sterile *Bactrocera dorsalis* and *Bactrocera correcta* male • Model for optimization of sterile fly release **Break** • Standard protocol for field evaluation of traps and bait stations • Novel attractants for fruit fly trapping • Development of new technologies to improve rearing, handling, monitoring and release systems in fruit fly SIT programmes • Development and optimization of infochemical-derived lures for monitoring *Anastrepha* fruit flies • Prototype for a Fruit Fly Decision Making System based on Electronic Traps	20 min 20 min 20 min 20min 20 min 10 min 20min 20 min 20 min 20 min 20min	Bishwo Mainali (Australia) Polychronis Rempoulakis (Australia) Preeaduth Sookar (Mauritius) N.T.T. Hien / H.T.K. Lien (Viet Nam) Pedro Rendon (Guatemala) Walther Enkerlin (Austria) Richard Alvarado (USA) Marta Martinez - Ignacio Pla (Spain) Julio Rojas / Pablo Liedo (Mexico) David Nestel (Israel)

	• Strengthen South Australia's fruit fly response program through a model-based adaptive management tool and targeted applied research	20 min	Katharina Merkel (Australia)
3 November Day 3 (9:00 – 11:30 pm am Vienna time)	• Use of a Trap Grid Computer Model to Optimize Trapping Networks • Development of "attract and kill" tools and analyzing SIT possibilities for fruit fly sustainable management in Burkina Faso • Introduction of wild genetic material in breeding colonies of genetic sexing strains for maintenance of high levels of genetic diversity and improvement of SIT • Presentation of the CRP proposal and adjustment of the Logical Framework Matrix (LFM), if needed • Presentation of the template for RCM reports.	20 min 20 min 20 min 60 min 30 min	Nicholas Manoukis (USA) Karim Nebie (Burkina Faso) David Haymer (USA) Walther (Austria) Walther (Austria)
4 November Day 4 (9:00 – 12:00 pm Vienna time)	• Drafting of the RCM report Note.- Split Sections of the report in working groups	180 min	All
5 November Day 5 (9:00 – 11:15 pm Vienna time)	Review of RCM report Finalizing RCM report Administrative issues with contracts and final comments	60 min 60 min 15 min	All Walther Rui

ANNEX 3 WORKING GROUPS

Working Groups by Research Topic

GROUP 1 PRODUCTION		GROUP 2 POSTPRODUCTION (SF Release + Supplements)	GROUP 3 FIELD OPERATIONS (Traps + BS)
GROUP 1a (GSS)	GROUP 1b (Diets)		
José Santiago /Salvador Meza*	Polychronis* Rempoulakis	Diego Segura*	Julio Rojas/Pablo Liedo*
Christian Morales/Edwin Ramirez	Christian Morales/Edwin Ramirez	Polychronis Rempoulakis	Nick Manoukis
Mariel Vanin	Mariel Vanin	Mariel Vanin	Karim Nebie
Valter Arthur/Thiago Mastrangelo*	Valter Arthur/Thiago Mastrangelo	Pedro Rendon	Pedro Rendon
David Haymer	Dori Nava	N.T.T. Hien / H.T.K. Lien	David Nestel Pla
Diego Segura	Carlos Pascacio*	Katharina Merkel	Katharina Merkel
Pablo Liedo	Preeaduth Sookar	Preeaduth Sookar	Preeaduth Sookar*
Carlos Caceres**	Martha Martinez/Ignacio Pla	Martha Martinez/Ignacio Pla*	Martha Martinez/Ignacio Pla
	Chris Weldon	Bishwo Mainlai	Bishwo Mainlai
	Bishwo Mainlai	Rui Cardoso**	Walther Enkerlin**

*Suggested group leaders

**IAEA support staff

ANNEX 4

ABSTRACTS

Improving Sterile Insect Technique and Field Components

Mendoza – Argentina

AUTHOR (S): Mariel Vanin*
ORGANIZATION: *Agricultural Sanitary and Quality Institute of Mendoza

SHORT SUMMARY OF PAPER

Abstract:

Argentina National Control and Eradication Fruit Fly Program is a good example of Integrate Pest Management under area wide concept.

The Program for the Control and Eradication of the Mediterranean Fly in the Province of Mendoza is operated by ISCAMEN – (Agricultural Sanitary and Quality Institute of Mendoza). It´s part of the national program of fruit flies PROCEM of SENASA and leads the use of the Sterile Insect Technique SIT and MIP since 1991, with more than 468 thousand hectares under official monitoring.

Following a strategy to enlarge the use of SIT and other environment friendly control system, a new facility was builded on 2007, the first multi-purpose facility in Latin America, with the potential to produce up to 700 million sterile pupae males per week. On 2019 ISCAMEN incorporated the chilled adult release system, creating a packing and release center in the south of the Province. Actually 100 million of adult flies per week are released under this technology on the free pest areas of Argentina (Mendoza and Patagonia).

The production of millions of sterile pupae involves supplying enough quantity of highly competitive sterile flies to the field, with excellent survival, compatible with the native wild flies of releasing zones. It´s well known the economic impact of larval diet on the pupal production cost. ISCAMEN´s facilities have been working specially to improve management of waste produced for exhausted larval diet could be improved with the development of gel diets.

The improvements of the insects released through the provision of nutritional supplements is a subject that concerns us, so we also introduce it to collaborate in its development.

Detection and monitoring systems is another of the components of high investment of human resources, for this reason we propose in this project the automation of these tasks through the development and integration of the existing communication computer platforms together with the intelligent systems in full development.

The incidence of urban conglomerates with a high supply of hosts and the low possibility of direct actions, for safety, health and privacy reasons represents a challenge for the program, therefore the integration of environmentally friendly control systems is proposed, as the use of the SIT, biological control and mass bait or trapping stations. The efficiency could be improved throughout some collaborative alliances with private owners. In this sense, the development of sterile biodegradable insect release devices is proposed, with an easy and practical handling for the owner. In parallel, we propose the development of communication systems through social networks that will increase the participation of the neighborhood, teenagers, students and teachers with the program at the same time to improve the integral sanitation of backyard´s hosts.

Improvement of the rearing and the genetic background of the Medfly genetic sexing strain

AUTHOR (S): José M. Esteban-Santiago, José S. Meza, Emilio Hernández-Ortiz, José Arredondo-Gordillo, Jorge Ibañez-Palacios, Maria F. Ruiz-Pérez, Yeudiel Gómez-Simuta, Marysol Aceituno-Medina, José P. Rivera-Ciprian, Reynaldo Aguilar-Laparra, José A. De la Cruz-De la Cruz and Maritza Juárez-Durán.

ORGANIZATION: National Program Fruit Flies SADER/SENASICA

SHORT SUMMARY OF PAPER

Abstract:

The Sterile Insect Technique (SIT) is a species-specific population management tool that has been developed for the pest control. Due Mexico suffers frequent incursions of *Ceratitis capitata* (Medfly), the national fruit fly campaign has implemented a permanent program that applies the SIT against this fruit fly pest on an ongoing basis in high-risk areas. As a result of area-wide integrated pest management using as main component the SIT, Mexico maintains its status as a Medfly free country. We are proposing to investigate the effect in the competitiveness of males by the insertion of a wild genetic base in the genetic sexing strain Vienna 8^{D53-} (GSS $V8^{D53-}$) produced in the mass rearing of Mexico facility, through a series of outcrossing with wild population of the region where we are applying the SIT. Also we will investigate about the differences larval diets, comparing different formulations of solid and liquid diets, different vegetal fibers and pelleted ingredients, in order to increase the quality and production. The intestinal microbiota of insects produced in mass rearing and wild ones, will be determine and if there are significant differences, we will try to re-establish their microbiota by differences methodologies and we will see the impact on field performance and longevity of sterile male. In addition, some live yeast probiotics will be tested to boost the performance of GSS V8D53- and finally, we will evaluate other newly developed GSS such as Vienna-8D53- FD_37 and T (X; 5).

Improving the Field Performance of *Anastrepha fraterculus* Sterile Males Through Specific Refreshing Protocols and Pre-Release Treatments

AUTHOR (S): Diego F. Segura[1,2], Francisco Devescovi[1,2], Silvina A. Belliard[1,2], M. Josefina Ruiz[2,3], Guillermo Bachmann[1,2], Lucia Goane[2,3], M. Teresa Vera[2,3] & Silvia B. Lanzavecchia[1].

ORGANIZATION: [1]Instituto de Genética "E.A. Favret", Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina; [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina, [3]Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán, Argentina.

SHORT SUMMARY OF PAPER

Abstract:

The SIT relies on the quality of laboratory reared, sterile males to survive under field conditions and sterilize wild females. Laboratory colonies usually experience genetic process such as genetic drift, unintended selection and bottlenecks that reduce their performance when the insects are released back to nature. Sterilization, through irradiation, sometimes contributes to a further reduction of the biological quality of sterile males. *Anastrepha fraterculus* is a major agricultural pest in South America. The development of environmentally safe control techniques, such as the SIT has been strongly promoted and intense basic research has been done to support the SIT. In this project, we aim at developing protocols that enhance the mating competitiveness and survival of sterile males under field conditions, as those found after release. Male enhancement will be attained by the adjustment of external factors such as the provision of dietary supplements (protein and hormones) and semiochemicals as part of a pre-release strategy. Based on previous findings, the field performance of sterile males will be significantly improved based on a series of pre-release treatments that includes the use of diets supplemented with protein and hormones, as well as the exposure to semiochemicals that increase the mating success of *A. fraterculus* males. We will also address the quality of different candidate strains for SIT, including a bisexual and a genetic sexing strain of *A. fraterculus*, and plan to introduce wild genes in order to improve the tolerance to different climates from cultivated areas affected by *A. fraterculus* in Argentina. The experiments proposed as part of the present project are intended to transfer knowledge about the reproductive biology and the genetics of *A. fraterculus* to SIT programs such as those planned in Argentina and Brazil. The final goal is to support to selection of a laboratory strain of *A. fraterculus* that enables the production of sterile males, sexually competitive with wild, fertile males, under the environmental conditions of those regions of Argentina in which the SIT is planned. We envision the design of appropriate protocols to increase sterile male field performance, making SIT more efficient.

Development and Evaluation of Genetic Sexing Strains for *Anastrepha fraterculus* To Enable Sterile Male-Only Releases in Brazil

AUTHOR (S): Valter Arthur & Thiago Mastrangelo

ORGANIZATION: CENA/USP

SHORT SUMMARY OF PAPER

Abstract:

The development of genetic sexing strains (GSSs) has enabled sterile male-only releases in the field to increase the efficiency and cost effectiveness of the Sterile Insect Technique (SIT) application (Franz et al., 2021). For *Anastrepha fraterculus* (Diptera: Tephritidae), the development of the first GSS based on *Y*-autosome translocations and using the genetic marker black pupae (*bp*) was reported by Meza et al. (2020). The GSS-89 showed to be the most genetically stable and productive strain after 8 generations under laboratory small-scale rearing. To evaluate the adaptation of this GSS to the rearing conditions in Brazil (Mastrangelo et al., 2021) and to verify its suitability for the MOSCASUL program (Kovaleski & Mastrangelo, 2021), a lot of pupae of the GSS-89 was imported by the CENA/USP in February 2020. The strain adapted to the new rearing conditions, but after four generations, a sharply increase in the number of unwanted recombinants in the colony was verified (Giustina et al., unpublished data). Currently, the cleaning of the mother colony is ongoing and single male families were set to restore the original characteristics of the GSS-89. To maintain the integrity of the sexing system and stability of the GSS, a filter rearing system (FRS) will be implemented at CENA/USP. After that, the GSS colony will be scaled up again for the conduction of a series of studies. In this context, the objectives of this project are: (1) to assess the quality control and productivity parameters of different *A. fraterculus* strains (including bisexual strains and GSSs); (2) to evaluate the mating compatibility between a GSS and different laboratory and wild *A. fraterculus* strains; (3) to determine the mating competitiveness of GSS males; (4) to demonstrate if the absence of sterile females would significantly affect the induction of GSS male sterility on a wild *A. fraterculus* population; (5) to improve the competitiveness and genetic diversity of the GSS by introducing wild gens; and (6) to assess the oviposition behavior of sterile females on commercial fruits.

Studies in bio-factories on Nutritional Larval Diets and Development and Maintenance of Genetic Sexing Strains of Two Species of Fruit Flies of Economic Importance

AUTHOR (S): Edwin Ramírez, Cristian Morales

ORGANIZATION: Medfly Program – Guatemala

SHORT SUMMARY OF PAPER

Abstract:

An essential component in the application of the sterile insect technique (SIT) for the control of pests such as fruit flies is the mass rearing of the target insect pests. Usually the economic investment for the operation of these bio factories is high; therefore, any efficiency achieved in its operational process has a direct impact on the convenience of using SIT.

Larval diets represent an important economic item in the cost structure of a mass rearing facility budget, also these diets can produce stability or instability of the production process depending to the quality of the diet ingredients. The prices of raw materials plus transportation costs, mainly for imported products, raise the costs of rearing insects. Therefore, studies for the development of nutritious diets with adequate costs and quality are of vital importance in the search for efficiency of the SIT.

Nutritional studies will be carried out to identify adequate food sources and bulking agents (including agar diets) that, due to their composition, promote a good performance of colony insects in the mass rearing of *Ceratitis capitata* and *Anastrepha ludens*. Also, to address this topic, the chemical composition of insects' preferred natural hosts will be characterized. During the first year, formulations of larval diets will be developed, where ingredients and percentages will be varied (including the use of gel diets). The response variables will be the quality of the insect in the larva, pupal and adult stage. The quality of the insects will be determined at the laboratory level and in field cages, following the procedures established on FAO-IAEA QC Manual. Also, the cost structure of the different formulations will be analysed.

Use of a Gelling/Texturing Agent to Replace Agar in Artificial Diet for *Anastrepha Fraterculus L*arvae, Aiming at The Sterile Insect Technique and Biological Control

AUTHOR (S): Dori Edson Nava

ORGANIZATION: Empresa Brasileira de Pesquisa Agropecuária, Embrapa Clima Temperado, Brazil

SHORT SUMMARY OF PAPER

Abstract:

The South American Fruit Fly *Anastrepha fraterculus* is the main fruit fly that causes damage to fruit crops in the Southeast and South regions of Brazil. Among the most attacked hosts are apple, peach, plum, citrus, vine and small fruits (blackberry, raspberry and blueberry). Damage caused by *A. fraterculus* can result in total fruit production loss if control measures are not adopted and the presence of this species limits the international fruit trade, especially for apple and citrus intended for fresh consumption. The management currently adopted is based on the monitoring and use of insecticides applied in the form of toxic baits and full area coverage. The main control target is the adults, but management became limited when systemic organophosphate insecticides were withdrawn from the market. Since then, the fruit sector has organized and sought to implement the MoscaSul Program with a focus on the use of the Sterile Insects Technique (SIT) and Biological Control (BC) with parasitoids. For this to be achieved, researches with *A. fraterculus* are being directed to solve the bottlenecks for the implementation of Moscasul. One of the main steps for the use of SIT and CB is the production of high quality insects with the lowest production cost. Among the ingredients, gelling agents are important to maintain the diet with the necessary quality so that the larvae can ingest and assimilate the other nutrients that are part of the diet composition. However, the most common gelling agent in these diets is agar, which has the disadvantage of its high cost, resulting in a mass rearing economically unfeasible. Thus, the aim of the project is to seek alternatives for the total or partial replacement of the agar gelling agent, used in artificial diets, by other products that can provide the same development to *A. fraterculus*, practicality in obtaining larvae, ease of purchase and that result in a lower production cost. The project has the following experiments: a) Larval development of *A. fraterculus* in semi-liquid artificial diets with different gelling agents, including: caraginine, xanthan gum, powdered gelatin, pectin and chia seeds (*Salvia hispanica*, Lamiaceae). For each possible product, preliminary experiments will be carried out before conducting the definitive experiment to assess the amount to be used and the form of use in the diet in harmony and adequate proportion with the other ingredients. In relation to insects, the number of larvae, number and weight of pupae, pre-oviposition period, fecundity, fertility and longevity will be evaluated. b) Definition of the diet to obtain second-instar larvae for *Doryctobracon areolatus* multiplication. A semi-liquid diet will be defined with the ingredients tested in the first experiment to obtain second-instar *A. fraterculus* larvae for *Doryctobracon areolatus* multiplication. c) Development of *A. fraterculus* and nutritional evaluation of a diet based on Chia seeds. d) Larval development of *A. fraterculus* in solid diets (pasty) without gelling agents. Studies will be carried out to obtain a solid diet for obtaining larvae aiming the sterile insect technique. e) Cost evaluation of diets and the potential use of co-waste. The cost of gelling/texturing agents and the possible destination of dietary waste will be evaluated, in a context of circular economy.

Development and Optimization of Gel Diet Rearing Systems for Improving the Sterile Insect Technique Against *Anastrepha ludens* and *Ceratitis capitata*

AUTHOR (S): Carlos Andrés Pascacio Villafán

ORGANIZATION: INECOL, Mexico

SHORT SUMMARY OF PAPER

Abstract:

An essential component in the application of the sterile insect technique (SIT) for the control of pests such as fruit flies is the mass rearing of the target insect pests. Usually, the economic investment for the operation of these bio factories is high; therefore, any efficiency achieved in its operational process has a direct impact on the convenience of using SIT.

Larval diets represent an important economic item in the cost structure of a mass rearing facility budget, also these diets can produce stability or instability of the production process depending to the quality of the diet ingredients. The prices of raw materials plus transportation costs, mainly for imported products, raise the costs of rearing insects. Therefore, studies for the development of nutritious diets with adequate costs and quality are of vital importance in the search for efficiency of the SIT.

Nutritional studies will be carried out to identify adequate food sources and bulking agents (including agar diets) that, due to their composition, promote a good performance of colony insects in the mass rearing of *Ceratitis capitata* and *Anastrepha ludens*. Also, to address this topic, the chemical composition of insects' preferred natural hosts will be characterized. During the first year, formulations of larval diets will be developed, where ingredients and percentages will be varied (including the use of gel diets). The response variables will be the quality of the insect in the larva, pupal and adult stage. The quality of the insects will be determined at the laboratory level and in field cages, following the procedures established on FAO-IAEA QC Manual. Also, the cost structure of the different formulations will be analysed.

Improvements for Rearing and Performance of Sterile Fruit Flies Through Manipulation of Dietary Lipids

AUTHOR (S): Christopher Weldon[1], John Terblanche[2] and C. Ruth Archer[3]

ORGANIZATION: [1]Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

[2]Centre for Invasion Biology, Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, South Africa

[3]Institute of Evolutionary Ecology and Conservation Genomics, University of Ulm, Germany

Correspondence: cwweldon@zoology.up.ac.za

SHORT SUMMARY OF PAPER

Abstract:

An individual's diet is a primary determinant of its fitness - survival, male attractiveness and female fecundity all depend critically on the amount and blend of nutrients that individuals consume. Optimising the dietary intake of sterile flies is therefore key to maximising the efficacy of SIT. However, this is challenging: recent work has shown that individual micronutrients can have a pronounced effect on phenotype and that nutrients interact to affect organismal performance. Further, different fitness traits may be optimised on diverse nutrient blends and optimal diets may differ between the sexes and age categories. The aim of this proposal is to characterise the relationship between nutrition and overall organismal performance in juvenile and adult *Bactrocera dorsalis*. We will focus on the effects of dietary sterols, which have striking phenotypic effects in vinegar flies, but whose impact on true fruit flies is unknown. Further, we will use a powerful dietary mapping approach to characterise how proteins, sterols and carbohydrates interact to affect juvenile development time and body size, female fecundity and survival and male pre- and post-copulatory reproductive performance. First, we will characterise the lipidome of adult and larval flies, to identify dietary sterols likely to be important for *B. dorsalis*. Second, we will test how candidate sterols affect traits of interest in a mass rearing context in both juvenile and adult flies. Finally, because different nutrients interact to affect phenotype, we will test how dietary sterols interact with other key nutrients (protein and carbohydrate) to affect juvenile development and adult male reproductive performance. In achieving this, we will optimise diets for mass rearing of *B. dorsalis* and promote the development of males that are actively favoured by females as mates to improve the efficacy of SIT. Our results will represent the basis for development of SIT against *B. dorsalis*, which is being actively considered for use in South Africa.

Enhancing Fruit Fly Sterile Insect Technique Through Improved and Cost-Effective Gel Larval Diet, Pre-Release Handling, and Monitoring

AUTHOR (S): Bishwo Mainali[1], Phil W Taylor[1], Vivian Mendez[1], Soo J Park[1], Terril Marais[2]

ORGANIZATION: [1] Applied Biosciences, Macquarie University, Sydney, NSW 2109, Australia

[2] Department of Primary Industries and Regions Government of South Australia, Port Augusta SA 5700, Australia

SHORT SUMMARY OF PAPER

Abstract:

Sterile Insect Technique (SIT) is promoted in Australia to manage Queensland fruit fly (Q-fly) *Bactrocera tryoni* (Froggatt), a major pest of horticulture in Eastern Australia, and a major biosecurity threat to other regions. Implementation of SIT requires a cost-effective system for production and delivery of high quality sterile male insects that can compete with wild males for matings with wild females, and an effective monitoring system. In pursuance of enhancing Q-fly SIT, this team is already well advanced in studies of gel larval diet, pre-release handling, and pheromone-based attractants with the ultimate goal of improved productivity, field performance and monitoring.

Work published by this team to date includes 1) development and refinement of gel diet 2) manipulation of ingredients of gel diet to reduce production cost 3) accelerated sexual development and improved field prevalence of Raspberry Ketone (RK) and Methoprene fed flies 4) enhanced field prevalence of sterile Q-flies through extended feeding and holding 5) identification of potential functions of endogenous compounds and their evaluation on various *Bactrocera* species. However, there is ample room for further refinement of the larval diet and pre-release diets for reduction in cost, and to expand on pheromone-based attractants. Over the coming 18 months we will seek to complete and publish the studies, especially on modification of gel larval diet and pre-release diets, and will continue exploring the positive and negative impacts of the changes in the diets on the performance of Q-fly.

We will assess performance of Q-fly reared on gel diets that will have gelling agent different from the original recipe and absence of nipagin. We will also evaluate quality control parameters including mating competitiveness of Q-flies fed on various proportions of yeast hydrolysate and plant-based protein. The findings will guide modification of gel larval diet and pre-release supplements and holding periods for more effective SIT.

Optimize Fruit Fly Production and Rear Out Systems, Improving Fruit Fly Management Practices, Enhance Fruit Surveillance and Control by Introducing Improved Trapping Systems and Decision-Making Tools for Management of Trapping Networks

AUTHOR (S): Polychronis Rempoulakis and Solomon Balagawi

ORGANIZATION: NSW Department of Primary Industries, Ourimbah, NSW 2258, Australia

SHORT SUMMARY OF PAPER

Abstract:

Queensland fruit fly, *Bactrocera tryoni* (Froggatt) and other minor species are among the most significant pest of Australia's $9 billion horticulture industry. The sterile insect technique (SIT) is considered a very effective management tool against this pest. Several improvements have been achieved during the last 5 years, but implementation of this method is reliant on adoption of the technical advances in operational setting. NSW DPI has been pioneering the SIT against Q-fly for the last 25 years, and is one of the major partners in the largest R+D projects in Australia, totaling more than 60M$. Here we propose to investigate a more holistic approach in management of fruit flies that will incorporate:

1) Further our knowledge in operational aspects of SIT, including collaborative efforts and technology tranfer to other partners

2) Enhance knowledge in field physiology of fruit flies that will inform best management practices.

3) Novel trapping systems including chemicals, lures and traps for effective monitoring and control of fruit fly population.

Improved Mass-Rearing Techniques for *Bactrocera dorsalis, B. zonata* and *Zeugodacus cucurbitae* (Diptera: Tephritidae)

AUTHOR (S): P Sookar, N Patel, S Raghoo, M Ramlugum, M Chumun, D Goorsohye

ORGANIZATION: Entomology Division

Ministry of Agro Industry and Food Security Mauritius

SHORT SUMMARY OF PAPER

Abstract:

The sterile insect technique has in many countries become an important control tactic for integration in area-wide integrated pest management programmes against fruit flies of economic importance. An important prerequisite of these programmes is the availability of adequate numbers of sterile male flies that are produced in large mass-rearing facilities. In Mauritius, the artificial larval diet for *Bactrocera dorsalis, B. zonata* and *Zeugodacus cucurbitae* is composed of sugarcane bagasse (6%), ground maize (6%), cane sugar (11%), waste brewery yeast (6%), wheat bran (6%), benzoic acid (0.1%), nipagin (0.1%), hydrochloric acid (0.008%) and water (64.8%). Good quality flies are produced in the newly constructed fruit fly rearing facility with percentage egg hatch, percentage emergence and percentage fliers above 77, 85 and 82, respectively. However, the main constraint of the conventional larval diet is the varying quality of the bulking agent (sugarcane bagasse) and the waste brewery yeast. Furthermore, there is a need for bulk storage and waste management. To solve these problems, studies will be carried out on the liquid and gel diets for the rearing of the fruit flies. The possibility of replacing commercial brewery yeast with waste brewery yeast in liquid and gel diets will be explored. To bring down diet cost, guar gum will be replaced with agar in gel diet. Endosymbionts (gut-associated bacteria) will be isolated from the flies and incorporated in larval diets to improve the fruit fly quality.

Influence of Dietary Protein on Performance of Sterile *Bactrocera dorsalis* and *Bactrocera correcta* Male

AUTHOR (S): Lien, H.T.K; Hien, N.T.T.; Thang D. D.

ORGANIZATION: Plant Protection Research Institute, Viet Nam

SHORT SUMMARY OF PAPER

Abstract:

The Oriental fly (*Bactrocera dorsalis*) and Guava fly (*Bactrocera correcta*) are among the most harmful fruit flies, which attack numerous fruit species including guava, orange, mango, guard, peach, Viet Nam has been suffering from pest problems relating to those flies for many kinds of fruit that have a high potential for exporting. Sterile insect technique is known as a friendly environmentally method. An important component of SIT method is a good mass rearing where adults are fed by diets which have major ingredients such as sugar and yeast at a suitable proportion. Some researches determined that adult's diet plays an important role in many activities in fruit fly life such as life-time, copulation ability, mating performance, sexing maturation and body-size of sterile fruit fly life. In the contrary, some studies have suggested that a large amount of protein may have detrimental effect on the flies. Although protein is essential for fruit fly development, the amount required to maintain balanced nutrition continues to be under consideration. In this project, aims include determining whether feeding diet at different proportion of yeast hydrolyzed would pose impact on the performance of flies such as changing in body size, survival, flight, dispersal of sterile fly of *B.dorsalis* and *B.correcta*. In this proof-of-concept 5-year project, the changing in body size and its impact on mating of sterile fly reared by different diets are determined via the activity that would be conducted in the 1st-2nd year of CRP. Coming to the 3rd year of CRP, the lifetime and sex maturation of sterile fly will be evaluated. And the flying assessment and dispersal ability will be tested during the 4th-5th year of CRP.

Improving the SIT Programme against *Ceratitis capitata* in the Valencian Community (Spain)

AUTHOR (S): Marta Martinez and Ignacio Pla

ORGANIZATION: TRAGSA Valencia, Spain

SHORT SUMMARY OF PAPER

Abstract:

The SIT programme against *Ceratitis capitata* has been operating since 2007 in the Valencian Community within an AW-IPM programme in more than 140.000 hectares. The quality of the sterile males released is a key factor for the success of a SIT programme.

This quality will depend directly on the rearing, handling and release processes and therefore any improvement on these will result in an improvement in the final quality of the released adults.

Although the different processes involved in the application of SIT have been improved over the years, several areas can be optimised by applying new technologies.

The objective of this proposal is to improve several aspects related to the implementation of an AW-IPM Programme with a SIT component to combat *Ceratitis capitata* in the Valencian Community:

• Mass-production of fruit flies enhanced using new and improved diets.

• Improving handling of sterile males through new rearing cages.

• Enhance field performance of sterile insects by providing protein in adult diet before releases.

• Development of a new system for both aerial and ground release.

• Development of an automatic trap for Medfly.

Development and Optimization of Infochemical-Derived Lures for Monitoring *Anastrepha* Fruit Flies

AUTHOR (S): Julio C. Rojas, Pablo Liedo, Leopoldo Cruz-López, Jorge Toledo & Edi A. Malo

ORGANIZATION: El Colegio de la Frontera Sur. Tapachula, Chiapas, Mexico

SHORT SUMMARY OF PAPER

Abstract:

Anastrepha fruit flies use volatile compounds emitted by males and fruits for searching mates and hosts. However, little has been explored about the use of these compounds for trapping of these fruit fly species. Trapping systems for fruit flies of the genus *Anastrepha* are limited to those that use food attractants, such as hydrolysed proteins or torula yeast, or synthetic lures derived from these baits, as Biolure ®. Parapheromones such as methyl eugenol, cuelure or trimedlure do not exist for *Anastrepha* and lures with such attractancy power are still highly desirable. The goal of this proposal is to develop and optimize lures derived from male and fruit volatiles for monitoring *A. ludens* and *A. obliqua*. The specific objectives of this proposal are: 1) To evaluate different blend volatiles from host fruits of *A. ludens* and *A. obliqua* reported in the literature to select the best performing lures in catching fruit flies, 2) To reduce the number of components of the original lures without losing their attractiveness, 3) To investigate the effect of ratio and concentration, and release rate of the components on the biological activity of selected lures, 4) To investigate whether the addition of male volatile enhances the attractivity of fruit volatiles derived lures, and 5) To compare the efficiency of optimized lures against the commercial proteinaceous bait used for monitoring *A. ludens* and *A. obliqua*. The biological activity of the blends will be evaluated in field-cage and field trials. Compounds and traps will be obtained from commercial sources. We expect to have optimized lures for each fruit fly species that can be more or as attractive than the proteinaceous baits.

In collaboration with the Mexican Fruit Fly Program, we will evaluate new lure formulations and new traps that could be more efficient for monitoring and control.

Prototype for a Fruit Fly Decision Making System based on Electronic Traps

AUTHOR (S): David Nestel and Victor Alchanatis

ORGANIZATION: Volcani Centre; Agricultural Research Organization, Israel

SHORT SUMMARY OF PAPER

Abstract:

During the last years, our group has been developing an electronic trap for fruit flies (FF) following the McPhail conventional trap. The ARO electronic e-trap is based on the principle of attracting and capturing fruit flies (specifically with male attractants), arresting the captured flies on a sticky board, snapping their image, and wirelessly uploading the image to the web for image analysis and further processing. The ARO e-trap and image analysis algorithm is now in an advanced developed stage, and a prototype has been successfully tested under the Horizon 2020 *FF-IPM* project. This e-trap prototype will be incorporated into a decision support system (DSS) within the framework of *FF-IPM* and used for testing improved surveillance system for invasive and expanding fruit flies. During the first period of the CRP we are proposing to improve the current ARO e-trap prototype by reducing energy use, and refining the electronics and mechanics of the prototype. Our objective during the next phase of the CRP is to share the gained experience in the use of the ARO e-trap in *FF-IPM*, and to develop plans for the application of DSS using the e-trap in a few selected specific pilot areas emerging from colleagues in the CRP. During the last phase of the CRP we expect to run the planned pilots to demonstrate the use of the technology, and evaluate its advantages.

Strengthen South Australia's Fruit Fly Response Program Through a Model-Based Adaptive Management Tool and Targeted Applied Research

AUTHOR (S): Katharina Merkel, Tom Kompas, Kym Perry, Terril Marais

SHORT SUMMARY OF PAPER

Abstract:

Real-world programs to manage fruit fly pests can be massive and complex with unpredictable events further challenging their success. This team will contribute to the CRP D41029, and the improvement of the application of sterile insect technique (SIT) and related technologies in an operational context through three major approaches: 1) evaluating alternative field management tools via laboratory and field studies, 2) operationalize scientific findings on improving mass rearing of sterile flies, and 3) model-based adaptive management. To enhance the suppression of target fruit flies, the team is assessing the performance of different tools. Both attraction and toxicity to fruit flies will be assessed using a series of assays that provide rapid and statistically powerful data for detecting fine differences in performance of bait formulations. The team will contribute to the reduction of production costs of mass rearing sterile *Bactrocera tryoni* by evaluating variants of larval gel-diets and pre-release diets. This research is based on findings by the team led by Prof. Phil Taylor at Macquarie University and will be conducted in collaboration. Finally, the team endeavours to build a functional holistic model to support decision making. We aim to improve fruit fly eradication by modelling suppression and monitoring scenarios and address questions such as: does the rate of released flies reach targeted overflooding ratios, how long to deploy interventions, and what is the eradication probability.

Our team is building on operational experiences from pest eradication and management programs, and the involvement in past and current research activities on applied management of fruit flies and other biosecurity threats.

Use of the TrapGrid Computer Model to Optimize Trapping Networks

AUTHOR (S): NC Manoukis

ORGANIZATION: USDA-ARS, Hilo Hawaii USA

SHORT SUMMARY OF PAPER

Abstract:

Trap networks, often including powerful semiochemical attractants, are essential components of fruit fly SIT programs. They enable assessment standing population pressure, of overflooding ratios needed for program goals, and QC information on sterile males under field conditions. However, quantitative assessment of trap network sensitivity and of the significance of trap capture patterns remains elusive. I will present the conceptual basis and operation of "TrapGrid" a computer simulation model that can be used to quantify capture probability. I will also share Mark-Release-Recapture field experimental methods used to parametrize the model and summarize results of experiments already conducted with three fruit fly species. Finally, I will suggest applications including improving the sensitivity and efficiency of trapping networks.

Development of "Attract and Kill" Tools and Analyzing SIT Possibilities For Fruit Fly Sustainable Management in Burkina Faso

AUTHOR (S): Mr Karim NEBIE

ORGANIZATION: Institut de l'Environnement et de Recherches Agricoles
BOBO-DIOULASSO BURKINA FASO

SHORT SUMMARY OF PAPER

Abstract:

Fruit flies constitute a major threat to horticulture in Africa and cause extensive economic losses. In Burkina Faso, the main fruit exported is mango which is the major fruit product. Following the detection of B. dorsalis in 2005, fruit damage has worsened and can reach worrying proportions. For example, the average rate of damage to the mango varieties Keitt and Brooks has been reported to reach 100% in the middle of the rainy season. Therefore, the implementation of fruit fly management programs with the use of GF-120, male annihilation, and application of various food baits is undertaken in mango orchards to control infestation by fruit flies. Unfortunately, despite the control methods deployed, the damage caused by fruit flies on mango remains a concern for small farmers. This situation can be explained by the fact that the control tools used are imported and are not within the reach of small farmers. To deal with these difficulties (accessibility and high cost of control products), it is necessary to prospect some control tools enhancing local resources including native plant extracts and the yeast waste that the Brassery company (BRAKINA) dump daily into nature. The last few years, INERA beneficed of rearing and trapping materials from AIEA allowing development of mass rearing system of B. dorsalis and gaining scientific data on fruit fly diversity, seasonal abundance, host plant and parasitoids. This project aims (i) to develop local fruit fly attract and kill tools, (ii) to improve mass production of B. dorsalis, (iii) to initiate laboratory studies as a prelude to the application of the insect sterile technique on the exotic species *Bactrocera dorsalis*.

Attract and kill tools will be made from native plant extracts and the yeast waste of brewery. Bioassays will be carried out to establish the optimal radiation dose of B. dorsalis pupa and to determine the dietary supplements to obtain male sterile of B. dorsalis of good biological quality.

Introduction of Wild Genetic Material in Breeding Colonies of Genetic Sexing Strains for Maintenance of High Levels of Genetic Diversity and Improvement of SIT

AUTHOR (S): David Haymer, Lorena Ruiz-Montoya, Salvador Meza and Pablo Liedo

SHORT SUMMARY OF PAPER

Abstract:

Mass reared colonies of insects can be impacted by intense selection and high levels of inbreeding. One solution for dealing with these issues is to introduce new genetic material into mass reared strains. In the past, however, the implementation of this solution has been limited by two major realities. First, it has been commonly seen that the first generation produced after injection of wild material may exhibit great vigor, but in the second generation, the colony may collapse and require considerable effort to be rebuilt to former levels of productivity. Second, even when the colony can be rebuilt, the tools have not been available to verify what new genetic material (if any) had been successfully introduced. To address these issues, we first developed tools to monitor the genetic health of a mass reared colony through calculation of a similarity index using RAPD genetic markers. This same index can also be used to document the impact of introducing new genetic material in any subsequent generation. In addition, the RAPD markers identified can also be used in the development of detailed genetic maps of chromosomes showing the locations of specific genes and other DNA based markers in the species undergoing mass rearing. These maps will be of great value in allowing for more precise monitoring of the fate of any new genetic material introduced into the colony. In this case, as new genes are identified as being responsible for desirable aspects of the phenotype needed for strain improvement, including those involved in mating behavior and other life cycle parameters, it will become more feasible to use these markers to guide the precise incorporation of this desirable genetic material into the mass reared strains to improve performance and enhance their effectiveness in the application of SIT.

ANNEX 5

Validation of Trapping Technologies Against Fruit Fly Pests

Standardized Protocol

W. ENKERLIN

FAO/IAEA Food and Agriculture Programme

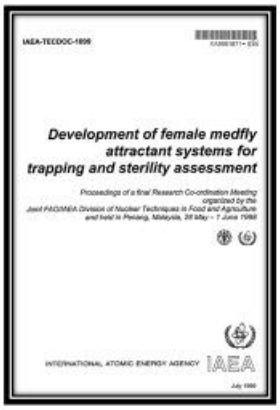
Objective

Validation of novel trapping technologies under different environmental and agroecological conditions using a standard methodology

Standard Methodology

1986 – 1992*

1993 - 1998



2000 - 2005

*R. Cunningham USDA, ARS, and A. Economopoulos, W. Klassen, D. Lindquist Joint FAO/IAEA Division

Trap Types and Attractants

Fruit fly trapping has become highly specialized

Materials

Fruit Fly Attractants for Validation:

BIOASSAYS	TREATMENTS
1 *Ceratitis capitata*	A = JT Plastic/TML 2 gm B = JT Plastic/Ceralure 2 gm C= JT Carboard/TML 2 gm (Control)
2 *Ceratitis capitata*	A = MLT/Biolure Unipack (Patch) B = Carousel Trap/Vial-Lure 3C C = Face IV Plastic/Biolure Unipack D = Face IV Plastic/Vial-Lure 3C E = MLT/Torula (Control)
3 *Anastrepha spp*	A = MLT/Torula Yeast + Shrimp Powder B = MLT/Vial 2C C = MLT/Torula (Control)

Methods

- **Plot Selection and Design**

✓ Areas with fruit fly populations at moderate levels
✓ Uniform agroecological conditions
✓ Within individual plots, the sites (blocks) where traps (treatments) are placed should be as uniform as possible.

- **Experimental Design**

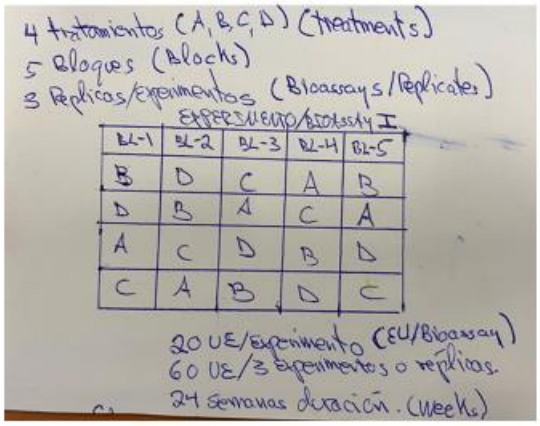
✓ Randomized complete blocks
✓ Five (5) blocks (I to IV) each with four treatments (A-D) equal to 20 Experimental Units (EU) per bioassay (see diagram in next slide)

Note.- The total EU will depend on the number of blocks and treatments in each bioassay.

✓ The bioassays will be replicated three times in different sites. In this case the total EU will be 60.
✓ Traps (treatments) should be rotated in each block every week after they are serviced.
✓ Each bioassay will be run for 24 weeks with weekly trap revisions
✓ Bioassays should be repeated every year (5 years)
✓ Depending on the type of attractant the replacement of the lures should be established:
  - Long lasting para-pheromones and synthetic food lures should not be replaced
  - Attractants with short life such as hydrolysate proteins (Torula) or other from natural extracts should be replaced every week.

✓ Depending on the type of trap, the replacement of the body of the trap should be established:

Experimental Design



Notes.-

1. The number of experimental units (EU) will depend on the number of treatments and blocks. Traps (treatments) should be rotated once per week in each block after all traps have been serviced.
2. Each experiment/bioassay should be replicated three times preferable in three different areas. The three replicates should run at the same time (simultaneously) for 24 weeks.

Methods

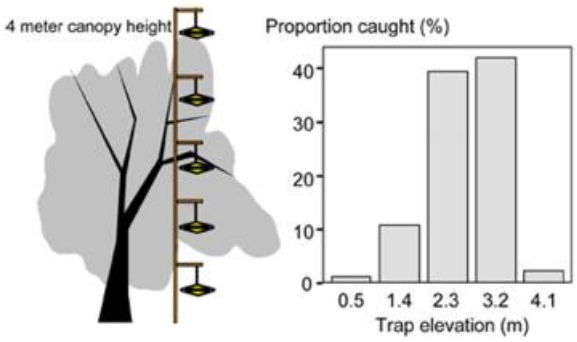
- **Trap placement**

✓ Traps should be placed on the mid to top part of the canopy of host trees (from 1 to 4 meters depending on the height of the tree)

✓ To the degree possible, traps within blocks should be placed in the same relative shade and position and in opposite direction to the dominant winds.

✓ All traps should be between 10 and 20 meters away from any other trap.

Selecting an appropriate trap site



Methods

- **Description of blocks**
  - Map (or croquis) of the blocks showing the host trees and the trap location (geographical coordinates)
- **Data for blocks and replicates**
  - Elevation (mosl)
  - Type of vegetation
  - Temperature (daily minimum and maximum)
  - Rain fall (mm)
  - Winds (direction and speed)
  - Maturation stage of the fruits in each block

Methods

- **Data collection**

✓ Traps will be checked once per week
✓ All traps should be checked the same day
✓ Record the number of males and females captured per trap
✓ Record of other species of fruit flies captured in traps
✓ Record the date and time when traps were checked
✓ Record the general condition of the trap at the time of the inspection

- **Data Analysis**

✓ Two-way analysis of variance using the data transformation $x' = \sqrt{x} + 0.5$
✓ Duncan multiple range test at 5% level will be run on all test data
✓ The coefficient of variation will be used to help determine if further testing is needed

Additional Information

❖ Treatments (traps and lures) should be defined

❖ Standard formats for data recording will be provided

❖ Based on the experimental design the amount of trapping materials to run the experiments should be assessed

❖ A calendar of bioassays needs to be prepared to organize the shipments of trapping materials