IAEA-D4.10.29-CR2
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REPORT

SECOND RESEARCH COORDINATION MEETING

Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture

Research Coordination Meeting on

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

15–19 May 2023

Reproduced by the IAEA

Vienna, Austria 2023

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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. SECOND RESEARCH CO-ORDINATION MEETING (RCM)

The second RCM was held in Vienna, Austria, from 15–19 May 2023. The list of participants is given in Annex 1. The agenda for the meeting is in Annex 2.

During the first two and a half days of the meeting RCM participants presented the results of the first year (18 months) research, as well as their research plans for the second year (next 18 months) of the CRP.

During the last two and a half days of the meeting, the CRP Logical Framework Matrix was review, discussions were held in working groups (Annex 3) to define the second year individual experiments and prepare the draft RCM report.

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 1.2 Diets (liquid, solid and gel)	1.1.1 Maintaining high genetic diversity through a novel genetic model and through genetic sexing strains 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 2.2 Sterile fly release model (decision support tool)	2.1.1. Supplements to enhance sterile male performance. 2.2.1 Excel model for sterile fly release optimization
3. Field Operations (Surveillance systems and control methods)	3.1 Optimization of surveillance systems 3.2 Control methods	3.1.1 Improved trapping systems (lures, traps and risk-based models) 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*	1.1 *A. fraterculus*		

	fraterculus to enable sterile male-only releases in Brazil			
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. Christopher 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	1.1 & 1.2 *C. capitata*		3.1 *C. capitata*

	and gel diets / Improved trapping			
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 *B. 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			3.2 *B. dorsalis, C. cosyra*

	sustainable management in Burkina Faso			
15. Julio Cesar Rojas Leon Mexico	Development and optimization of infochemical-derived lures for monitoring *Anastrepha* fruit flies			3.1 *A ludens, A obliqua*
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*	2.1 *B. tryoni*	3.1 *B. tryoni*
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

A brief summary of the specific research being conducted by each CSI including the research topic, a brief description and goals is presented in the table below.

NAME CSI	FRUIT FLY SPECIES	TYPE OF RESEARCH	BRIEF DESCRIPTION	GOALS
Edwin Ramirez	*Ceratitis capitata*	Artificial rearing (larval and adult diets) GSS	Alternative ingredients Torula yeast vs soybean Genetic diversity of mother colonies and males. Introduce and evaluate new GSS.	Optimization of mass rearing Cost reduction Improve the performance of steril males in the field. Evaluate the yield and quality of the new GSS.
David Haymer	*Ceratitis capitata*	Analysis of genetic diversity	Evaluate genetic markers for determining levels of genetic diversity	• Assess levels of genetic diversity in bisexual and strains used in GSS colonies during maintenance and for refreshment/introduction of new genetic material
Carlos Pascacio	*Anastrepha ludens*	Artificial rearing (larval diets)	Alternative gelling agents (calcium-alginate) Larval density in the diet. Design of experiments and response surface methods	Advance the state of knowledge related to rearing tephritid fruit flies on artificial diets and move towards a mechanistic understanding of gel diet rearing systems for *Anastrepha ludens*
Preeaduth Sookar / Savitree Raghoo	*Bactrocera dorsalis, B. zonata, Zeugodacus cucurbitae*	Artificial rearing (larval diets)	Alternative ingredients Different mixture formulation of commercial yeast and waste brewery yeast used. Use of agar in gel diets Wheat germ oil vs canola oil	Optimization of mass rearing

Thiago Mastrangelo	*Anastrepha fraterculus*	New GSS Artificial rearing	Evaluation of production and quality parameters Alternative ingredients	Availability of a GSS for male only releases Optimization of mass-rearing procedures Up-to-date knowledge of sexual compatibility between different populations.
Sandro Nörnberg	*Anastrepha fraterculus*	Artificial rearing (larval diets)	Alternative ingredients (gelling and bulking agents)	Optimization of mass rearing Cost reduction
Diego Segura	*Anastrepha fraterculus*	New GSS Artificial rearing (larval diets) GSS sterile male performance	Evaluation of production and quality parameters Genetic stability Mating performance	Assessment of GSS-89 as potential strain to implement SIT against *A. fraterculus* Optimization of larval diets for the GSS
Teresa Vera	*Anastrepha fraterculus*	Artificial rearing (larval diets)	Alternative ingredients Wheat germ vs corn flour	Optimization of mass rearing Cost reduction
Mariel Vanin	*Ceratitis capitata*	Artificial rearing New GSS (FDF)	Alternative ingredients Cost and parameters on different formulation of standard diet Evaluation of production and quality parameters	One alternative ingredient for gel diet Cost reduction on new formulation New GSS FDF offer reduction of the cost of management of the colonies in the facility
Salvador Meza	*Ceratitis capitata*	New GSS	Evaluation of production and quality parameters	
Mata Martínez	*Ceratitis capitata*	Artificial rearing	Bulking agent alternative to sugar beet pellets. Design of gel diet.	Increase the larval recovery rate and stabilise production throughout the year. Reducing the waste after exhausting the diet.

Bishwo Mainali	*Bactrocera tryoni*	Artificial diet (larval and adult)	Alternative gelling agents; cost-effective plant and dairy based protein sources	Development of cost-effective larval and adult diets for Q-fly SIT without compromising the fly quality
Katharina Merkel	*Bactrocera tryoni*	Cost-benefit analysis	Cost-benefit analysis	Cost reduction
Chris Weldon	*Bactrocera dorsalis*	Artificial diet (larval and adult)	Alternative ingredients (lipids)	Optimisation of mass-rearing

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 systems will allow for the creative possibility to cross in mass numbers of females that typically carry specific phenotypes used as markers. By carefully following an appropriate crossing 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 maintain an appropriate level of genetic diversity. It is 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, and to then validate the production and quality profile of the resultant offspring, including mating competitiveness, in 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 mating behaviours needed to compete for mating with fertile wild males in target areas subject to control. Insect colonization could affect those specific patterns and behaviour due to laboratory colonies being maintained in different artificial environments. Insects adapted to laboratory conditions, as in other domesticated organisms, lose their heterozygosity in a few generations. Tropical fruit flies present very complex courtship behaviours with males aggregating in mating arenas or leks, 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 genetic composition of the olive fly, *Bactrocera oleae*, changed drastically in the first five generations of adaptation under laboratory conditions (Zygouridis et al, 2014).

Hybridization of the laboratory-adapted insects with wild ones and the selection process for specific traits has demonstrated that the insect could preserve specific characteristics during several generations (McInnis et al 2002). However, those characteristics are not permanent due to the selection process under laboratory conditions forcing the colony to return to 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 breeding 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.

A standard protocol for evaluation for refreshment and/or introduction of new genetic material into the GSS colonies is needed.

A standard set of protocols are needed for evaluation of parameters for larval growth under mass-rearing conditions and quality control measurements relating to mating success/competitiveness in the field for each of the species using GSS colonies.

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 two 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).

Summary of Consolidated Results/Achievements by 2 RCM

The use of genetic markers (such as RAPDs) to monitor levels of genetic diversity in bisexual and mass reared genetic sexing strains (GSS) was initiated for *Ceratitis capitata*, *Anastrepha ludens*, *Anastrepha fraterculus* and *Zeugodacus cucurbitae*.

Identification of genetic markers potentially linked to phenotypes of interest such as male specific genes and male mating success commenced.

Importation and evaluation of new genetic sexing strains in terms of stability and productivity under small scale and mass rearing conditions in different facilities was started.

4.1.2 Artificial Rearing

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

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 approach of experimentation and modelling 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.

Summary of Consolidated Results/Achievements by 2 RCM

Yeast still seems to be the best option for providing nutrients to larvae in comparison with other sources that have been tested. However, to reduce the cost of this larval diet ingredient, soybean meal can be used to replace yeast by as much as 50% by weight.

Gel diets based on agar may be a viable option for mass-rearing when cheaper, food-grade agar sources are available. Carrageenan, or a blend of xanthan and locust bean gum, are also a viable gelling agent for diets that are more affordable than agar in many circumstances. In the case of calcium-alginate, although it has the advantage of gelling at room temperature, this gel does not seem to be a viable option for mass-rearing due to the low yields it produces. Solid bulking agents are still suitable in mass-rearing facilities when consistent supply and quality is reliable in certain regions of the world. For example, sugarcane bagasse is used effectively in Mexico for *C. capitata*. But encouraging results were obtained when sugarcane bagasse was substituted with cellulose as a bulking agent in the mass-rearing diet of *C. capitata* in Mexico. Corn bagasse, apple pulp, wet okara and citrus pulp are promising alternatives to sugar beet pellets for *C. capitata* in Spain but tests need to be performed at a larger scale.

A relevant factor that has been little addressed but that has a great impact on fly production and quality is that of larval density in the diet.

For all of the options tested to date, detailed cost-benefit analyses need to be performed to identify the best available combination of options.

1.2 POSTPRODUCTION PROCESS

A brief summary of the specific research being conducted by each CSI including the research topic a brief description and goals is presented in the table below.

NAME CSI	FRUIT FLY SPECIES	RESEARCH TOPIC/SUBTOPIC	BRIEF DESCRIPTION	GOALS
Bishwo Mainali	*Bactrocera tryoni*	Supplements	Alternative protein source for adult flies before field release • Yeast hydrolysate (YH), Mubarqui diet (MD) and sugar only	Improve adult fly quality; reduce operational cost
Mariel Vanin	*Ceratitis capitata*	Supplements Sterile Fly released model	Evaluation of different food for adutI pre releasing Adapt to local conditions Rendon release model	Incremental of survival and recaptures after releasing Adjustment of releasing rates. Improvement on the permanence of sterile fertile ratios in the field. Reduction of costs and/or incremental of benefit
Diego Segura	*Anastrepha fraterculus*	Supplements Semiochemical exposure	Identification of semiochemicals that can be used to increase the mating	Development of pre-release diets that combine protein and methoprene

			success of sterile males Assessment of methods to deliver methoprene included in the adult diet	Development of practical alternatives to expose males to compounds that stimulates their sexual behavior
N.N.T. Hien	*Bactrocera dorsalis* *Bactrocera correcta*	Supplements	Evaluation different proportion of hydrolysed yeast in adult diet to find out the suitable diet that will be mixed with methyl eugenol to rear sterile fruit fly before release	Enhance the performance of sterile fruit fly in mating success to support to SIT
Katharina Merkel	*Bactrocera tryoni* *Ceratitis capitata*	Release technology	Evaluation of past release rates using SIT density calculations spreadsheet (IAEA)	Optimization of SIT release rates
Marta Martinez	*Ceratitis capitata*	Supplements Emergence cage Release machine	Evaluation of Mubarqui diet compared to sugar only. Evaluation of HY plus sugar and egg protein plus sugar compared to sugar only. Design of a new emergence	Improve the sterile male mating performance. Incorporate the use of a new emergence cage to improve the working conditions maintaining the sterile males quality.

			cage prototype. Design of a new release system prototype.	Reduce the cost of male releases and achieve a versatile system to be used in aircrafts and ground vehicles.
Chronis/Solomon Balagawi	*Bactrocera tryoni and Zeugodacus cucumis*	Supplements	Research to evaluate the suitability of mubarqui diet to improve mating propensity of B. tryoni and Z. cucumis was undertaken.	Research goal was to improve mating competitiveness of B. tryoni and Z. cucumis
Sookar Preeaduth	*Bactrocera dorsalis* *Zeugodacus cucurbitae*	Release methods	Evaluation of the release of sterile flies with the ground release machine	To improve the release of sterile flies for SIT programs.

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 achieving the final objectives of an operational programme. This system requires the proper handling of the flies after irradiation. The processes involved include insect emergence, pre-release holding and 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 investments made to insect production and shipping 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 potential 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 (USA), (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).

Recently, the use of drones for the aerial release of sterile flies is being considered as an innovative technique. Using drones for the aerial release of sterile flies offers several advantages such as precision and coverage and efficiency and cost-effectiveness over the conventional aerial and ground release practices. However, it comes with some limitations too such as payload capacity and may require adapting the technique to the specific requirements of the target tephritid species and local environmental conditions for optimal results.

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
ludens	MSRM	CESSNA 206	7 Million	Mexico
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 disadvantage is 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 bags is labour-intensive, making it a very costly system. Moreover, an 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 adult 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. Of note, the chilling process of the sterile flies can lead to a decrease in quality parameters, especially if very long chilling times are required due to the distance between the rear-out facilities and the release areas. In any case, the 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.

As aforementioned, the recent interest on use of drones for the release of sterile releases while is appealing, the limitations such as payload capacity, flight range and endurance, and regulatory frameworks are potential issues that require attention before the wide-scale use of the technology.

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.

Summary of Consolidated Results/Achievements by 2 RCM

- Aircraft and ground vehicles: The studies on feasibility of utilizing ultralight aircraft, including both aerial and ground vehicles with low weight and low fuel consumption, for medfly sterile insect programme led to selection of six appropriate light aircraft models. Furthermore, potential of a prototype capable of releasing high-quality medfly males using these light aircraft and ground vehicles was successfully designed, assembled, and tested.

- Fly Density Model and release density: Data collected from a comprehensive survey of hosts in urban, semi-urban, and rural areas has been organized into a user-friendly database. Further, gathered and verified detailed information about the available hosts in each area, including urban, semi-urban, and commercial locations. To estimate various factors, the Rendon model adopted in combination with local meteorological data.
- Significant improvements made in data collection methods to facilitate easier manipulation and analysis of the gathered information from the pest free areas in Australia.

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, Solomon Balagawi

Background Situation Analysis

The SIT relies on the quality of factory reared sterile males to survive under field conditions and sterilize wild females. Production facility 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 analogues 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 increases 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.

Yeast hydrolysate (YH), which contains protein, carbohydrates, fiber, sterols, vitamins, and minerals, serves as a valuable component of the adult diet in the mass-rearing and rearing out of various tephritid flies, Although YH is commonly used, it is expensive and can constitute up to 90% of the total cost related to the adult diet in mass-reared fruit flies. To reduce production and rear-out costs, there has been interests in economical alternative sources of protein and other essential nutrients for tephritid flies. However, up until now, there have been limited achievements in developing a cost-effective pre-release supplement.

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 applied 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 to enhance performance of the SIT programmes. 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 on 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 with the addition of 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 large-scale conditions of action of 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.
- 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 required to express their maximum reproductive potential while balancing important trade-offs between sexual performance and survival.

Summary of Consolidated Results/Achievements by 2 RCM

- A commercial adult diet (MUBARQUI) has been tested for *B. tryoni, C. Capitata* and *Z. cucumis*. For *B. tryoni,* MUBARQUI diet failed to support male reproductive organs development. Additionally, MD did not improve mating propensity for B. tryoni and Z. cucumis. Both the Mubarqui diet and sugar only diet had similar effect on the development. For *C. capitata,* Mubarqui diet was able to improve male mating competitiveness compared to sugar only diet. However, it negatively affected longevity and flight ability.

- Alternative protein sources have been compared to YH in terms of flight ability, mating propensity, longevity. and reproductive organ development for *B. tryoni* and *C. capitata*. None of the protein sources tested so far showed promising results.
- For *B. dorsalis* and *B. correcta* adding YH to the adult diet contributed to sexual maturation and reduced mortality of sterilized males. The proportion of YH to sugar can be reduced to 1:4 without compromising the quality of the produced sterile males.
- Acceleration of sexual maturation by feeding males an adult diet supplemented with methoprene has been confirmed for *A. fraterculus*, although the effect is lower than that of topical treatment suggesting that males are not receiving the same dose.
- Two individual volatile compounds capable of stimulating the calling behavior of *A. fraterculus* males have been identified.
- A new emergence cage for sterile medfly males has been designed and tested with positive results.

4.3 FIELD OPERATIONS

A brief summary of the specific research being conducted by each CSI including the research topic, a brief description and goals is presented in the table below.

NAME CSI	FRUIT FLY SPECIES	TOPIC/ SUBTOPIC OF RESEARCH	BRIEF DESCRIPTION	GOALS
Julio Rojas	*Anastrepha spp*	Surveillance	• Commercial food attractants • Fruit volatiles (white sapote and yellow chapote)	• Improve trapping systems
Teresa Vera	*A. fraterculus*	Surveillance	• Commercial food attractants • Alternative pheromone and para-pheromone attractants (Epianastrephin and dimethyl)	• Improve trapping systems
Chronis Rempoulakis	*B. tryoni*	Surveillance	• Adoption of TrapGrid model	• Improve trapping systems
Marta Martínez	*C. capitata*	Surveillance	• Design and test a smart trap	• Improve trapping systems
Karim Nebie	*B. dorsalis* *C. cosyra*	Surveillance	• Test efficacy of different attractants and traps	• Improve trapping systems
Mariel Vanin	*C. capitata*	Surveillance	• Interaction between captures and hosts	• Improve trapping systems

			• Evaluation of traps and attractants	
Nick Manoukis	*B. dorsalis, C. capitata, A. ludens, Z. cucurbitae*	Surveillance	• Distribution and adoption of TrapGrid	• Application of TrapGrid model • Improve trapping systems
Savitree Raghoo	*B. dorsalis, Z. cucurbitae*	Surveillance	• Evaluation of traps and attractants • Adoption of TrapGrid	• Improve trapping systems
Thiago Mastrangelo	*A. fraterculus*	Surveillance	• Evaluations of attractants	• Improve trapping systems
Katharina Merkel	*C. capitata, B. tryoni*	Surveillance	• Adoption of TrapGrid	• Improve trapping systems
David Nestel	*C. capitata, B. zonata*	Surveillance	• Evaluate new smart trap prototype attractiveness and function	• Improve trapping systems
Nick Manoukis	*(various)*	Decision Support Tool	• Application and parameterization of TrapGrid model	• Improve trapping systems • Uptake of TrapGrid (standardization)
Mariel Vanin	*C. capitata*	Decision Support Tool	• Application and parameterization of TrapGrid model	• Improve trapping systems
Preeaduth Sookar (Savitree Raghoo)	*B. dorsalis B. zonata Z. cucurbitae*	Decision Support Tool	• Application and parameterization of TrapGrid model	• Improve trapping systems
Polychronis Rempoulakis	*B. tryoni*	Decision Support Tool	• Application and parameterization of TrapGrid model	• Improve trapping systems
Katharina Merkel	*B. tryoni C. capitata*	Decision Support Tool	• Application and parameterization of TrapGrid model • Develop a decision Support Tool	• Improve trapping systems • Support decision making in action programs

David Nestel	*C. capitata*	Decision Support Tool	• Development and evaluation of a DSS	• Improve trapping systems • Support decision making in action programs
Karim Nebie	*C. cosyra* *B. dorsalis*	Control methods	• Development of local food and pheromone attractants	• Reduce horticultural production costs • Facilitate organic farming
Preeaduth Sookar	*Bactrocera zonata* *Z. cucurbitae*	Control methods	• Evaluation of food-based bait station using IAEA field evaluation protocol	• Improve suppression of target fruit flies
Mariel Vanin	*C. capitata*	Control methods	• Evaluation of bait station in urban and peri-urban areas	• Improve suppression of target fruit flies
Katharina Merkel	*C. capitata*, *B. tryoni*	Control methods	• Evaluated commercially available alternatives to Naturalure • Develop Standard protocol for laboratory and greenhouse trial	• Improve suppression of target fruit flies • Support the assessment of new tools

4.3.1 Improved Trapping Systems (Traps and Attractants)

Participants: David Nestel, Julio Rojas/Pablo Liedo, Valter Arthur/Thiago Mastrangelo, Martha Martinez/Ignacio Pla, Preeaduth Sookar Polychronis Rempoulakis* /Solomon Balagawi, Teresa Vera/Lucía Goane.

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.
- Adoption of 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.

Summary of Consolidated Results/Achievements by 2 RCM

- Potential compound blends for the attraction of *A. obliqua* and *A. ludens* have been identified. Optimization of these blends is currently investigated.
- Attraction to synthetic sexual pheromones has been shown for *A. fraterculus* (morphotype 1) in field cage experiments. Field evaluation of food-based attractants for *A. fraterculus* showed different levels of attraction in different eco-regions (Argentina and Brazil).
- Smart-trap prototypes based on image recognition for the detection of *Ceratitis capitata, Bactrocera zonata* and *Bactrocera dorsalis* have been assessed, and electronic and mechanic modifications to initial designs are currently under investigation. In addition, a conceptual prototype to distinguish between sterile and wild *C. capitata* is under development to support management programs.
- Deployment strategies have been tackled from two directions: i) from the perspective of trap attractiveness and ii) from the perspective of the landscape risk. Deployment modelling based on trap attractiveness has been improved through the inclusion of more factors, such as diffusion and its relation to environmental conditions. Workshops were held to support the application for field data and implementation is ongoing. Regarding the deployment based on the landscape risk studies are being implemented in several regions to select better strategies for operational programs. The use of an application to manage deployment of traps has been initiated.
- To develop decision support tools, key outputs need to be identified in consultation with key stakeholders. Scoping studies can clarify regional requirements for outputs and identify the availability of data.
- The assessment of attractants has been followed the standard "FAO/IAEA protocol for trap evaluation" (Annex 5).

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 need 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 known 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.

Summary of Consolidated Results/Achievements by 2 RCM

- Recent progress regarding parameters for TrapGrid analyses have focused on the pest movement component of the model.
- A published study by Caton et al. (2021) includes estimates of the diffusion coefficient *D* for *Bactrocera dorsalis, Ceratitis capitata,* and *Anastrepha ludens*. Miller et al. (2022) have tracked the movement of individual *Zeugodacus cucurbitae* via harmonic radar and produced parameters that can be used for the more realistic “random correlated walk” version of the movement model when simulating that species.
- In terms of accessibility of the model to researchers and programme managers, a recently published book chapter includes a detailed description of how to use the model (Manoukis 2023) and should help with efforts by others to quantify trap capture.

- A deployment strategy developed in FF-IPM is being evaluated at this stage. Data derived from these field studies is currently being analysed, and conclusions will be incorporated into a Decision Support System being developed for this aim.

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 other 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 are 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., entomopathogens), 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.

Summary of Consolidated Results/Achievements by 2 RCM

- Following the evaluation standard protocol for bait stations provided by IAEA, attraction to Pestman and Cerati pack baits by *Z. cucurbitae* was shown to outperform alternative baits.
- Brewery yeast waste demonstrated the potential to attract eleven fruit fly species belonging to five genera (*Bactrocera, Ceratitis, Dacus, Perilampsis, Zeugodacus*) with a bias toward female attraction.
- Laboratory and greenhouse trials demonstrated the potential of alternative baits (Natflav and Anamed) to Naturalure to control *C. capitata*.

LOGICAL FRAMEWORK

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

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. 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 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. 3. Optimize fruit fly surveillance and control by introducing improved trapping systems and decision-making tools for management of trapping networks and bait stations.	Improved production volumes and insect quality Enhanced sterile fly quality indices and sterile fly release parameters Sensitivity and management of trapping networks improved. Increased effectiveness in population suppression.	Reports, protocols and published papers. Reports, decision making models and published papers. Reports, decision making models and published papers.	Managerial support and availability of expertise and resources required to conduct large-scale applied research in mass-rearing and irradiation. Managerial support and availability of expertise and resources required to conduct large-scale applied research to improve sterile male performance and aerial release. Managerial support and availability of expertise and resources required to conduct large-scale field experiments to improve surveillance systems and population suppression.

Outcomes (Results) 1. Improved sterile insect technique through more efficient mass-rearing methods and improved GSS strains. 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.	Increased yields and improve sterile fly quality indices. Production protocols available. Enhanced sterile fly quality indices and sterile fly release parameters Decision making tool for aerial release of sterile insects available. Sensitivity and management of trapping networks improved. Increased effectiveness in population suppression.	Technical reports, published papers. Protocols adopted. Technical reports, published papers. Sterile fly density model adopted. Technical reports, published papers. Trapping models adopted.	Improved technologies, improved GSS and tools adopted by MS. (**same as above**) Improved technologies and tools adopted by MS. (**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 Reports and / or published papers	Methods and resources for QC assessment available Managerial support to adopt the model
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	Methods and resources to measure trap efficiency available

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	Managerial support to adopt the model
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	Methods and resources to measure FTDs and fruit infestation available
3.3 Fruit fly control improved through more efficient population suppression tools	Increased efficiency for population suppression measured through FTD and fruit infestation index.	Journal special issue with published scientific papers.	Data and articles for publication available
4. Results published in a peer reviewed journal.	Papers drafted and submitted.		

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.Preparation of necessary research protocols by 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 was held from 15 to 19 May 2023 in	Procurement orders available	Progress satisfactory.

	Vienna, Austria		
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 and population suppression	Participants and RCM Progress Reports.	Methods and resources available.
13. Organize final RCM to assess the success of the CRP in	4th RCM to be held 2Q 2026.	Participants and RCM Final Reports	Final reports are submitted to the Agency.

reaching its objectives and review the final publication. (2Q, 2026) 14. Evaluate the CRP and submit evaluation report. 15. Publish the results of the CRP in a special issue of a peer reviewed journal.	Satisfactory completion of research agreements and technical contract At least 20 publications accepted.	Report Scientific publication s.	Contracts and Agreements properly managed by counterpart organizations. Methods and resources available. Consensus can be found on appropriate peer review journal and acceptance by journal obtained.

V. PUBLISHED ARTICLES IN THE FRAMEWORK OF THE CRP

Topic 1 – Production

Castro-López, C., Pascacio-Villafán, C., Aluja, M., García, H. S., González-Córdova, A. F., Vallejo-Cordoba, B., & Hernández-Mendoza, A. (2022). Safety assessment of the potential probiotic Bacterium *Limosilactobacillus fermentum* J23 using the Mexican fruit fly (*Anastrepha ludens* Loew, Diptera: Tephritidae) as a novel *in vivo* model. Probiotics and Antimicrobial Proteins, 1-16.

Guillén, L., Pascacio-Villafán, C., Osorio-Paz, I., Ortega-Casas, R., Enciso-Ortíz, E., Altúzar-Molina, A., ... & Aluja, M. (2022). Coping with global warming: Adult thermal thresholds in four pestiferous *Anastrepha* species determined under experimental laboratory conditions and development/survival times of immatures and adults under natural field conditions. Frontiers in Physiology, 2103.

Pascacio-Villafán, C., Righini, N., Nestel, D., Birke, A., Guillén, L., & Aluja, M. (2022). Diet quality and conspecific larval density predict functional trait variation and performance in a polyphagous frugivorous fly. Functional Ecology, 36(5), 1163-1176.

Pullock DA, Malod K, Manrakhan A and Weldon CW (2023) Larval and adult diet affect phenotypic plasticity in thermal tolerance of the marula fly, *Ceratitis cosyra* (Walker) (Diptera: Tephritidae). Frontiers in Insect Science 3: 1122161.

Topic-2 Post-Production

Belliard, Bachmann, Fernández, Hurtado, Vera, Segura. 2022. Identification of host plant volatile stimulants of *Anastrepha fraterculus* male courtship behaviour. Frontiers in Ecology and Evolution. Volume 10, https://doi.org/10.3389/fevo.2022.943260

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ANNEX 1

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ANNEX 2

AGENDA

SECOND RESEARCH COORDINATION MEETING

Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture

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

Room M7 Vienna International Centre
Vienna, Austria

15 - 19 May 2023

Project Officer: Walther Enkerlin

Monday, 15 May 2023

09:00 – 09:10 **Rui Cardoso & Walther Enkerlin** – Welcome and Objectives.

09:10 – 09:30 Introduction of participants.

09:30 – 09:45 **Elena Zdravevska -** Administrative matters (if any) and uploading of presentations.

09:45 – 10:15 **Walther Enkerlin** – Review of Agenda, meeting procedures and current status of the CRP.

SESSION I: Production Process (Artificial Rearing and Genetic Sexing Colonies)

Presentations by participants (Chairperson: Cristopher Weldon)

10:15 – 10:45 **Edwin Ramirez** – "Studies in Biofactories on Nutritional Larval Diets and Development and Maintenance of Genetic Sexing Strains of Two Species of Fruit Flies of Economic Importance".

10:45 – 11:00 *Coffee Break*

11:00 – 11:30 **Savitree Raghoo** – "Improving rearing and control techniques with the integrated use of SIT for *B. dorsalis*, *B. zonata* and *Z. cucurbitae*". (**Focus: Mass rearing**)

11:30 – 12:00 **Sandro Nornberg –** "Developmen of *Anastrepha fraterculus* in diets with different gel and bulking agents".

12:00 – 13:00 *Lunch Break*

13:00 – 13:30 **Thiago Mastrangelo** - "Updates on the Research Activities in CENA: Preliminary results with the GSS-89 and evaluation of different food lures for *Anastrepha fraterculus*". (**Focus: Mass rearing**)

13:30 – 14:00 **Carlos Pascacio** – "Agar and Carrageenan Gel Larval Diets Outperform Calcium-Alginate-Based Diets for Rearing *Anastrepha ludens* (Diptera: Tephritidae), a Fruit Fly Pest Controlled Trough the Sterile Insect Technique".

14:00 – 14:30 **Christopher Weldon** – "Improvements for rearing and performance of sterile fruit flies through manipulation of dietary lipids: Characterisation of important sterols and fats".

14:30 – 15:00 **Teresa Vera** – "Improving trapping procedures and mass rearing of *Anastrepha fraterculus*". (**Focus: Mass rearing**)

15:00 – 15:30 *Coffee Break*

15:30 – 16:00 **Mariel Vanin** – "Improving Sterile Insect Technique and field components Mendoza – Argentina". (**Focus: Improvement of mass rearing for *Ceratitis capitata***)

16: 00 – 16:30 **Marta Martinez** – Improving the SIT Programme against *Ceratitis capitata* in the Valencian Community (Spain). (**Focus: Mass rearing**)

16:30 – 17:00 **Salvador Meza** – "Improving rearing and genetic background of the genetic sexing strain of Medfly" (Virtual presentation)

Tuesday, 16 May 2023

SESSION I (cont'd) Production Process (Artificial Rearing and Genetic Sexing Colonies)

09:00 – 09:30 **David Haymer** – "Use of RAPD markers to monitor colonies of mass reared insects and improve the prospects for successful application of SIT".

SESSION II: Post-Production (Supplements and sterile fly release model)

Presentations by participants (Chairperson: Teresa Vera)

09:30 – 10:00 **Bishwo Mainali** – "Comparison of Mubarqui diet with yeast hydrolysate as a potential Queensland fruit fly adult diet"

10:00 – 10:30 **Diego Segura Argentina** – "Improving the field performance of *Anastrepha fraterculus* sterile males through specific refreshing protocols and pre-release treatments."

10:30 – 11:00 *Coffee Break*

11:00 – 11:30 **Marta Martinez** – "Improving the SIT Programme against *Ceratitis capitata* in the Valencian Community (Spain)" (**Focus: Release prototype, emergence cage and adult diet**)

11:30 – 12:00 **Mariel Vanin** – Improving Sterile Insect Technique and field components Mendoza – Argentina (**Focus: Rendon model**)

12:00 – 13:00 *Lunch Break*

13:00 – 13:30 **Hien N.T.T. – "**Influence of dietary protein on performance of sterile *Bactrocera dorsalis* and *Bactrocera correcta* male".

13:30 – 14:00 **Solomon Balagawi – "**Optimisation of Queensland fruit fly production and rear out systems." (**Focus: Suitability of the Mubarqui diet for Q-fly)**.

SESSION III: Field Operations (Surveillance systems and control methods)

Presentation by participants (Chairperson: Chronis Rempoulakis)

14:00 – 14:30 **Teresa Vera** - "Improving trapping procedures and mass rearing of *Anastrepha fraterculus*". **(Focus: Trapping procedures)**

14:30 – 15:00 **Julio Rojas** – "Development and optimization of infochemical-derived lures for monitoring *Anastrepha* fruit flies".

15:00 – 15:30 *Coffee Break*

15:30 – 16:00 **Karim Nebie** – "Development of fruit fly attractants based on brewery yeast wastes in Burkina Faso".

16:00 – 16:30 **Savitree Raghoo** - "Improving rearing and control techniques with the integrated use of SIT for *B. dorsalis*, *B. zonata* and *Z. cucurbitae*". (**Focus: Trap evaluation**)

16:30 – 17:00 **Thiago Mastrangelo** - "Updates on the Research Activities in CENA: Preliminary results with the GSS-89 and evaluation of different food lures for *Anastrepha fraterculus*". (**Focus: Trapping**)

17:00 – 17:30 **Mariel Vanin** – Improving Sterile Insect Technique and field components Mendoza – Argentina (**Focus: Smart traps and trapping grid model**)

Wednesday, 17 May 2023

SESSION III (cont'd) Field Operations (Surveillance systems and control methods)

09:00 – 09:30 **Nicholas Manoukis** – "Quantifying insect trap network captures using TrapGrid". (Virtual presentation)

09:30 – 10:00 **Marta Martinez** - "Improving the SIT Programme against *Ceratitis capitata* in the Valencian Community (Spain)". (**Focus: Smart traps**)

10:00 – 10:30 **David Nestel** – "Redesigned E-Trap and Development of a Trap Deployment Strategy for Fruit Fly Surveillance".

10:30 – 11:00 *Coffee break*

11:00 – 11:30 **Katharina Merkel – "**Strengthen South Australia's fruit fly response program through a model-based adaptive management tool and targeted applied research".

11:30 – 12:00 **Chronis Rempoulakis** – "Optimisation of Queensland fruit fly production and rear out systems." (**Focus: pilot sterile Q-fly field release program**)

12:00 – 13:00 *Lunch Break*

SESSION IV: Working groups: discussion, planning, and coordination of work plans (Chairperson: Walther Enkerlin and Group Leaders)

13:00 – 13:30 General discussion on individual research workplans for the second-year experiments (next 18 months) and composition of the working groups**.**

13:30 – 17:00 Conformation of working groups. Discussion, planning, and coordination of work plans

<u>Thursday, 18 May 2023</u>

SESSION IV (cont'd):

09:00 – 10:00 Working groups: discussion, planning, and coordination of work plans

10:00 – 10:30 *Coffee Break*

10:30 – 12:00 Working groups: discussion, planning, and coordination of work plan

12:00 – 13:30 *Lunch*

13:30 – 15:00 Working groups: discussion, planning, and coordination of work plan

15:00 – 15:30 *Coffee Break*

15:30 – 17:00 Revision of the CRP documents (introduction, individual proposals) including planning of the activities to carry out for the next 18 months

Friday, 19 May 2023

SESSION V: Review of the CRP documents, drafting and compiling the RCM report (Chairperson: Walther Enkerlin)

09:00 – 10:00 Drafting RCM report and preparation of the list of results/achievements

10:00 – 10:30 *Coffee Break*

10:30 – 12:00 Compiling RCM report and the list of achievements

12:00 – 13:00 *Lunch*

13:00 – 16:00 Presentation of the 2nd RCM report.

16:00 – 16:15 ***Closing***

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)**		
Salvador Meza	Edwin Ramirez	Diego Segura*	Chronis Rempoulakis*
Edwin Ramirez	Mariel Vanin	Chronis Rempoulakis	Julio Rojas
Mariel Vanin	Thiago Mastrangelo	Mariel Vanin	Nick Manoukis
Thiago Mastrangelo	Sandro Nornberg	N.T.T. Hien	Karim Nebie
David Haymer	Carlos Pascacio	Katharina Merkel	Thiago Mastrangelo
Rui Cardoso**	Savitree Raghoo	Savitree Raghoo	Katharina Merkel
	Martha Martinez	Martha Martinez	David Nestel
	Chris Weldon*	Bishwo Mainlai	Katharina Merkel
	Bishwo Mainlai	Solomon Balagawi	Savitree Raghoo
	Teresa Vera	Daguang Lu**	Martha Martinez
	Katharina Merkel		Bishwo Mainlai
			Teresa Vera
			Walther Enkerlin**

*Suggested Chair

**IAEA support staff

ANNEX 4

ABSTRACTS

SESSION I: Production Process (Artificial Rearing and Genetic Sexing Colonies)

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, Amilcar Gutierrez, Pedro Rendón

ORGANIZATION: Medfly Program – Guatemala

Abstract

In the cost structure of the mass rearing of the sterile male fruit flies, the larval diet represents one of the highest percentages (around 30%), occupying second place, only after the highest cost (staff salaries). Consequently, any efficiencies achieved in larval diets have a high impact on improving the SIT within control programs.

One of the objectives set for this CRP was to increase the efficiency in the mass rearing of sterile males of *Ceratitis capitata* and *Anastrepha ludens* by characterizing nutritional components for insect rearing and developing or modifying larval diet formulations. These studies have become more relevant today due to the unusual increase in the costs of raw materials and transportation.

In the diet formulations used for the mass rearing of *C. capitata* and *A. ludens* larvae, torula yeast is the most expensive ingredient and is present in the highest percentage (around 50 % of the total dry ingredients). Therefore, to have a greater impact on the efficiency of mass rearing, our study aimed to review the percentage of torula yeast in the larval diet. During the evaluations the percentage of torula yeast was reduced and was compensated with soybean meal, which provides an adequate amount of protein (51.46%), slightly higher than the amount provided by torula yeast (48.48) and has a lower cost. The study was carried out in small, medium, and large-scale rearing, maintaining the nutritional component of the insects, the quality of the produced sterile males, and the rearing costs as analysis variables. The substitution of the percentage of torula yeast for soybean meal was done gradually, starting with a substitution of 35% of torula yeast and then 50%. The results showed that the replacement of torula yeast with soybean meal is viable, both in 35% and 50%. Even with the substitution of 50% of torula yeast, the number of larvae produced (liters of larvae/kilogram of diet) is the same (without significant difference, 0.168 and 0.164 for treatment and control, respectively). Quality (pupa weight, % emergence, % fliers, longevity) was measured in the rearing laboratory and at the release center. Sterile males reared on larval diets with substitution of torula yeast for soybean meal showed no significant difference in quality parameters compared to those males reared on diets without yeast substitution. In conclusion, the reduction of the percentage of torula yeast and its respective substitution with soybean meal is viable in the mass rearing of insects and represents an increase in the efficiency of SIT for control programs.

Improving rearing and control techniques with the integrated use of SIT for B. dorsalis, B. zonata and Z. cucurbitae

AUTHOR (S): S.Raghoo, P.Sookar

ORGANIZATION: Entomology Division, Ministry of Agro-Industry and Food security, Mauritius

Abstract

The melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) is a major pest of cucurbitaceous vegetables. About 30 to 100 % losses may occur, depending on the cucurbit species and the season. The sterile insect technique is an important control tactic in area-wide integrated pest management programmes against these fruit flies of economic importance. The SIT involves the release of large numbers of mass -reared sterile males over defined areas, where they mate with the wild females resulting in no offspring and a declining pest population. 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%). This larval diet has many drawbacks and to overcome these problems, liquid and gel diets have emerged as promising alternatives. In this study, a liquid diet for rearing melon flies was developed. An assessment of the cost effectiveness of SIT was performed. The experiment was designed to evaluate main (waste brewer yeast vs. commercial brewer yeast), and interactive effects (waste brewer yeast + commercial brewer yeast combinations) of the yeast sources on production and quality parameters of fruit flies. The first trial resulted in a low pupal recovery and no conclusive results obtained. The experiment shall be repeated.

Trapping is a key component of programs against Tephritids. Mass trapping is being used to suppress the pest population and mixtures of protein as food attractant with chemical products such as parapheromones have been used. They help to reduce the fruit flies population and control crop infestation. This experiment is carried out to study the attractancy and efficiency of the different attractants namely: Success (GF-120) Fly O bait, Pestman, FF-240, modified waste brewery yeast and Ceratipack®. 300 ml of the baited food attractant were placed in the PET bottles. Ceratipack® was included as another treatment for comparison. The traps were placed 1m above ground level. The traps were serviced on a weekly basis and the attractants renewed. The attractancy of the traps were evaluated by calculating the FTD for males and females respectively. A preliminary trial was carried out in cucurbits field at Mont-Ida and L'Esperance Quartier Militaire. A Statistical analysis; ANOVA followed by Tukey test showed a significant difference between Ceratipack®, Pestman and the other treatments.

Development of *Anastrepha fraterculus* in diets with different gel and bulking agents

AUTHOR (S): Sandro Daniel Nornberg[1,], Leticia Jansen Medeiros[2], Rafael da Silva Gonçalves[1], Daniel Bernardi[2], Adalecio Kovaleski[3], and Dori Edson Nava[4]

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Abstract

The use of artificial larval diet for mass-rearing *Anastrepha fraterculus* has led to the development of basic to applied research, such as biological control programs and the use of the sterile insect technique. In Brazil, significant progress has been made in respect to the artificial rearing of *A. fraterculus* using the diet proposed by Salles (1999), who uses agar as a gelling/texturizing agent. Despite the rearing in this diet that allow the production of a high number of flies, they must be optimized to increase insect yields and decrease production costs. In order to lower production costs, the percentage of agar used in the diet was reduced by more than 50%. However, the cost is still high. Thus, experiments were carried out with the use of different gelling/texturizing agents to replace the agar. Initially, preliminary tests were carried out to adjust the amount of different products (chia seeds, xanthan gum, carrageenan, gelatin, pectin and the control agar) in Salles' diet. Biological parameters were evaluated: weight and number of pupae, percentage of emergence, sex ratio and percentage of flying insects. As a result, it was observed that the diet containing carrageenan in place of agar generally presented (considering all parameters) the best values. In a second moment, a definitive experiment was carried out evaluating the same biological parameters of the preliminary test in the parental generation (F0) and in the descendants (F1). As a result, it was also observed that the diet containing carrageenan provided a better development of *A. fraterculus*. For the F1 generation, it was not possible to set up the experiment for insects from diets containing gelatin and pectin due to the lower percentage of emergence. Diets with chia seeds and xanthan gum should be better studied, as they showed intermediate values. Biological parameters related to longevity and fecundity are still being evaluated. During the experiments, it was not possible to evaluate the F3 and F5 generations due to problems that occurred during breeding. Thus, the experiment to evaluate these gelling/texturizing agents is being repeated.

Assessment of the Efficacy of Different Food Lures for the Recapture of Sterile Flies from the South American Fruit Fly

AUTHORS: Henrique Martinelli, José Bressiani, Caio Neri, Valter Arthur, Thiago Mastrangelo.

ORGANIZATION: CENA/USP

Abstract

The South American fruit fly, *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae), is one of the main pests of fruit trees in Brazil. To know the efficiency of the different types of existing commercial attractants and finding new lures is essential for the field monitoring of this pest and other fruit fly species. Therefore, two release-recapture experiments were conducted in a mango orchard (without fruits) to evaluate the efficiency of major commercial food lures used in Brazil and a prototype lure from *Colly* (p-Colly) for trapping and monitoring of *A. fraterculus*. The assessed attractants in Test-I were a hydrolysed protein of animal origin (CeraTrap™) and 4 proteins of plant origin: BioAnastrepha, IscaTradiconal (IST), a new formulation of Isca Vittia (IN), and the Dismel protein. In Test-II, only CeraTrap™ and p-Colly were compared. McPhail traps were baited with 300 mL of each lure. The trials were carried out in a randomized complete block design, with 5 blocks per treatment in Test-I and 12 blocks per treatment in Test-II. In both tests, the distance among replicate blocks (orchard rows) was 20 m, and the distance among traps within blocks was 30 m. The traps were placed in the inner part of the plant canopy, at 1.5 m above ground level. Traps were rotated weekly to prevent any bias in treatment location. Insect inspection and sequential rotation were performed every 7 d, together with replacement of lures or refill of the evaporated volume in the treatments with CeraTrap™ or p-Colly. Response variables included the number of flies trapped and rate of adult capture (flies per trap per day - FTD). The number of times the weekly capture rate exceeded the traditional threshold of 0.5 FTD for each lure was also evaluated. In Test-I, 1.2% of the released flies were recaptured (130 out of 10,700 sterile flies), and the total number of adults captured by the lures CeraTrap™, BioAnastrepha, IST, IN and Dismel were 71, 37, 6, 8, and 8 adults, respectively, over a period of 28 days. In Test-II, 43% of the released flies were recaptured (6,205 out of 14,425 flies), and CeraTrap™ lured *A. fraterculus* in amounts above the economic threshold (0.5 FTD) over 80% of the study period, whereas p-Colly lured the same species in amounts above the control level for 60% of the same period. Traps baited with CeraTrap™ presented greater capture rates in both studies.

Agar and Carrageenan Gel Larval Diets Outperform Calcium-Alginate-Based Diets for Rearing *Anastrepha ludens* (Diptera: Tephritidae), a Fruit Fly Pest Controlled Trough the Sterile Insect Technique

AUTHOR (S): Carlos Pascacio-Villafán [1], Luis A. Caravantes-Villatoro [1], Ixchel Osorio-Paz [1], Larissa Guillén [1], Hugo S. García [2], Erick Enciso-Ortíz [1], Alma Altúzar-Molina [1], Roxana Barran-Prior [1] and Martín Aluja [1]

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Abstract

Research on the development and optimization of artificial diets is key to the progress of the Sterile Insect Technique. Here, we used Design of Experiments and Response Surface Methods to study physicochemical and nutritional characteristics of gel larval diets developed for rearing the tephritid fruit fly pest *Anastrepha ludens*. We also assessed fly production and quality parameters, as well as diet consumption and nutritional traits of larvae and adults, as a function of the gel type (agar, carrageenan, and calcium-alginate), the gel content (in coded units from -1 to +1), the larval density in the diet (in coded units from -1 to +1), and their double interactions. Calcium-alginate diets were firmer, more resistant to penetration and less acidic than the agar and carrageenan diets. Overall, the larval recovery, pupation, pupal weight, and adult emergence of *A. ludens* were lower in calcium-alginate diets compared to the agar and carrageenan diets. Results point to a higher diet and protein consumption per larva in the calcium-alginate diets, and to interaction effects between the gel type and the larval density in the diet, and between the gel type and the gel content in the diet, on carbohydrate and lipid intake per larvae. The excretions of larvae from the calcium-alginate diets had high levels of lipids and uric acid, but low levels of ammonia when compared to the excretions of larvae from the agar and carrageenan diets. Protein levels in the excretions of larvae from the calcium-alginate diets were higher than those of larvae from the agar and carrageenan diets at low levels of gel content in diets but were the lowest at the highest level of dietary gel content. Larval density in the diet had positive, negative and curvilinear effects on the production and quality parameters of flies evaluated; diet consumption by larvae; protein, ammonia and uric acid content in larval excretions; and carbohydrates, lipids, and protein content in larvae. We discuss the results regarding the development and optimization of larval diets to resemble as closely as possible the developmental environment experienced by larvae within a host fruit in nature.

Improvements for rearing and performance of sterile fruit flies through manipulation of dietary lipids: Characterisation of important sterols and fats

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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 project 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 and house flies, but whose impact on true fruit flies is unknown. In this study, we characterise the lipidome of adult and larval flies, to identify dietary sterols likely to be important for *B. dorsalis*.

In a pilot study, we collected ten 10-day old adult female and male *B. dorsalis* from a laboratory culture held at the University of Pretoria, which were snap-frozen using liquid nitrogen. Each fly was weighed, and dried in an oven at 50˚C to constant weight to obtain dry weight and to calculate water content. To each fly, 0.5 ml of a 1:1 chloroform methanol solution was added. To five females and males, 30 µg of 5-a-cholestane was added (as a sterol standard), and 30 µg of octacosane was added to the remaining flies (as a fatty acid standard). Each fly was left in the solvent for 12 hours, homogenised, dried, then couriered to Canisius College for further processing and quantification of lipids. The samples were resuspended and saponified. Sterols were conjugated to iodotrimethylsilane (TMSI) and freed fatty acids were converted to fatty acid methyl esters before structures were verified by gas chromatography-mass spectrometry (GCMS). Initial and dry weight, and water content of females tended to be slightly higher in females and males but these differences were not significant. Focusing on sterols usually encountered in insects, we detected cholesterol, sitosterol and the steroid cholestan-3-one, in increasing quantities on a dry weight basis. Important fatty acid methyl esters were methyl palmitoleate, oleic acid methyl ester, palmitic acid methyl ester in similar amounts, and significantly less stearic acid methyl ester. The detection of relatively high levels of cholestane-3-one in *B. dorsalis* adults is surprising because it is relatively rare in plants and insects. An exception is the wood wasp *Sirex noctilio*, where it has been suggested that high levels of this compound in body tissues are not due to intake of fungi on which it feeds but rather the activity of gut bacteria.

We are currently running additional experiments to expand on the results reported above. In particular, we are rearing *B. dorsalis* larvae in fruit (guava) and standard carrot-based larval diet (containing brewer's yeast) that have an intact microbial community or one that has been depleted (by removal of the egg chorion). Third-instar larvae, newly-emerged adults, and 10-day old adults fed on only sugar or a complete diet of sugar and hydrolysed yeast are being harvested for further analysis of body lipids. These results will be compared with the lipids available within the diet. By doing so, we can disentangle the contribution of diet and the vertically transmitted gut microbial community to the lipidome of larval and adult *B. dorsalis*. In future, 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.

Improving trapping procedures and mass rearing of *Anastrepha fraterculus*

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ORGANIZATION: [1]Facultad de Agronomía, Zootecnia y Veterinaria, UNT, Argentina. [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. [3]INTA Argentina. [4]Instituto de Genética "Ewald A. Favret", GV-IABIMO, Argentina. [5]ARS-USDA, USA.[6]SENASA Argentina.

Abstract

This proposal aims to improve field trapping procedures and nutrition protocols for mass rearing of the South American fruit fly, *Anastrepha fraterculus*. At present, monitoring of this cryptic species complex relies on food-based attractants. We evaluated six commercially available attractants in commercial fruit orchards from two agro-ecological regions in Argentina during the summer of 2022. The efficacy of the attractants differed according to the region indicating the need to evaluate them in each particular case. In addition, given the poor selectivity of food-based attractants, we determined the attractiveness of two lactone semiochemicals (epianastrephin, and the synthetic analog g-lactone, (±)-trans-tetrahydroactinidiolide, dimethyl) through electroantennography and behavioral tests carried out under field cage conditions. *Anastrepha fraterculus* Brazilian 1 morphotipe males and females of different physiological conditions were evaluated. Although all physiological conditions showed a positive antennal response, attraction was higher for virgin females and mature males. This is the first report that clearly shows a role of epianastrephin in this *A. fraterculus* morphotipe associated with its reproductive status. Moreover, dimethyl showed a promising performance since it elicited the same fly's response than epianastrephin; requires less steps to be synthesized and could be a good early-detection attractant particularly in pest free areas such as entry ports of importing countries. Consequently, we propose for the next period to evaluate its attractiveness to other *Anastrepha*'s species and members of the cryptic species complex as well as to run a dose response experiment in open field conditions in order to determine the adequate formulation. Release-capture experiments will also allow determining the attraction distance of the lure. With respect to rearing protocols, we propose to evaluate different larval diet formulations in conjunction with the mass rearing facility from Mendoza, Argentina, which recently initiated the upscaling of a laboratory strain. Parameters associated with immature development, nutrient content at emergence, and reproductive capacity of males and females will be determined. Lastly, in the context of this CRP, we will present recent advances in the evaluation of *A. fraterculus* capacity to cope stress. Results and proposals for the next step will be discussed in the context of their relevance for the SIT.

Improving Sterile Insect Technique and field components Mendoza – Argentina

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ORGANIZATION: Agricultural Sanitary and Quality Institute of Mendoza

Abstract

The production of sterile insects is essential to reach the necessary quantity and quality to carry out efficient actions, with the objective of obtaining the status of free area for the Province. We worked with TSL-VIENNA 8 and TSL-FDF; Egg/Pupal recovery percentage and eggs hatched were evaluated under rearing for release colony and male production. The average was 54.32 and 29.51, and an eggs hatch of 80.70% and 54% respectively. The parameter of wrong sex in male was on average 0.05%. Wild males and females were evaluated in the field with sterile males TSL-Vienna 8 and TSL-FDF with/without chilling system. The variables: number and proportion of copulations, RSI, duration and mating latency showed similar statistical results between both strains. The habitual larval diet is in continuous improvement, tests of gel diets have been incorporated, prioritizing the use of locally produced agents with promising results. The response variable % egg/pupa recovery showed values between 18.76% and 57.7%. The best results were: dehydrated mashed potatoes (P5) and the combination of Xanthan Gum and Guar (XG2). The adult diet used was also analyzed, comparing the use of liquid and solid protein against sugar and water, showing better parameters for the solid option. In the field monitoring, alternatives for male and female with different attractants were evaluated. The partial results showed significant differences only in females. It is expected to evaluate the results using the TrapGrid model to deterinmine the probability of capture of the insect. For smart traps, the Jackson type was successfully evaluated in the 2022 Emergency Plan. It is expected to incorporate MP type devices in a next stage. The release densities are planned by the Rendon Method. The frequency adjustment incorporates the results of the study on the number of generations according to grades/day for rural and urban areas. The planning of communication strategies and actions are focused on urban areas to strengthen the commitment of the community, allowing the development of actions such as the census by images of domiciliary hosts.

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

AUTHOR (S): Marta Martínez Gonzalvo

ORGANIZATION: TRAGSA

Abstract

The SIT programme against *Ceratitis capitata* has been operating since 2007 in the Valencian Community within an AW-IPM programme in over140.000 hectares. The quality of the sterile males released is a key factor for the success of SIT programmes.

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.

In recent months, several improvements have been implemented in our SIT programme. We have developed new technologies in the areas of mass rearing, emergence, release and trapping of sterile males of *Ceratitis capitata.* The most notable goals we have achieved are:

- Design of different new larval diet formulations to be tested.
- Design and assembly of a first release prototype valid for ultralight aircrafts and ground vehicles.
- Design and assembly of a first emergence cage prototype.
- Evaluation of the Mubarqui diet in adults of *Ceratitis capitata*.
- Development of image detection and classification models using deep learning techniques to achieve an automatic system for counting adult individuals of *Ceratitis capitata*.
- Initial design of an automatic trap for the capture, identification and counting of medfly adults in the field.

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

AUTHOR (S): José M. Esteban-Santiago, José S. Meza, José P. Rivera-Ciprian, Reynaldo Aguilar-Laparra, Trinidad Artiaga-López, Eduardo Hidalgo-Mayorga, Jorge Ibañez-Palacios, Yeudiel Gómez-Simuta and Maritza Juárez-Durán.

ORGANIZATION: Programa Operativo de Moscas, SADER/SENASICA-IICA

Abstract

México was declared free of the Mediterranean fruit fly, *Ceratitis capitata* (Medfly) since 1982, however, due to the continuous incursions of the pest, a permanent action program has been established to monitor the entire Mexican territory and prevent its establishment. Within this program, the sterile insect technique (SIT) is a central component to control the pest in areas with detections. To apply SIT, a mass rearing facility has been established to produce and release about 500 million sterile males per week. The success of this production depends largely on the artificial diet used (larval diet) to transform the eggs of these insects into viable larvae capable of pupating and giving rise to healthy, competitive adults.

In this study, we modified the larval diet formulation for *C. capitata* strain Vienna 8, trying to improve and explore the low cost of protein source, antimicrobial agent and texturizing agent. We tested in separate trials the protein source provided by inactivated yeast (current) versus soybean meal, the antimicrobial nipagin (current) versus formalin and the texturizer corn stover (current) versus cellulose, all experiments at a semi-massive level (1,500 kg/replicate). Formalin diets showed higher larval yields than nipagin diets, however, lower larval weights, pupal weights and post-irradiation adult emergence were obtained. No difference was found in pre-irradiation adult emergence, flying adults and survival. Cellulose compared to corn stover showed no difference in the amount of larvae recovered, but significantly higher larval weights, pupal weights and adult survival percentages were obtained, although there was a slight decrease in emerged and flying adults. Experiments comparing the soybean meal *vs.* yeast diet showed that soybean cannot replace yeast, but it can be used in combination. When yeast was above 50% of soybean in the combination, larval yields remained similar, with a slight decrease in larval and pupal weights.

On the other hand, two genetic refreshment protocols for Mexican fruit flies were tested (single-fase refreshment and continuous refreshment) and the Vienna-8D^{53}-FD_37 and T (XX;55) new GSS were received from the IPCL of sieberdorf, Austria to start their evaluation. And finally, random amplified polymorphic DNA (RAPD) was performed to determine the genetic variability in the laboratory population of *C. capitata* compared to the wild population.

Use of RAPD markers to monitor colonies of mass reared insects and improve the prospects for successful application of SIT

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Abstract

For several Tephritid species of interest for mass rearing and SIT (sterile insect technique), we have identified RAPD (randomly amplified polymorphic DNA) genetic markers that can be used to assess the genetic makeup of the mass reared colonies and various wild populations of these same species. We have used this method on populations of *Ceratitis capitata, Zeugodacus cucurbitae, Anastrepha ludens and Anastrepha obliqua* to compare levels of similarity within and between populations. The ability to monitor and assess the genetic makeup of colonies of these species is essential to avoid problems associated with the loss of genetic diversity and inbreeding in mass reared colonies, and to develop a framework for monitoring the impact of the introduction of new genetic material in the colonies. Another goal of this project is to identify markers within the genome of each species that may be linked to phenotypes such morphological markers, the sex of the flies and/or behavioral traits such as mating success.

SESSION II: Post-Production (Supplements and sterile fly release model)

Comparison of Mubarqui diet with yeast hydrolysate as a potential Queensland fruit fly adult diet

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Abstract

Yeast hydrolysate (YH) plus sugar (1:3) is currently the standard adult diet for mass-rearing and pre-release rear-out of Queensland fruit fly (Q-fly) *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) for sterile insect technique (SIT) programs, but YH is a costly protein source. As a potential alternative to YH, this study compared the commercial 'Mubarqui' diet (MD) (currently used for Mexican fruit fly SIT programs) with YH and sugar-only (SD) diets in terms of key quality parameters for both production and pre-release rearing. Q-flies fed on MD and SD did not produce any eggs, indicating that MD and SD are unsuitable for Q-fly mass production. There was no difference in the total consumption of MD and YH, but the flight ability of the MD- and SD-fed flies was better than the YH-fed flies. Simulating the current practice of 5d holding of Q-flies at the rear-out centre, we conducted another experiment, in which the flies were sorted out by sex and provided with either MD, YH, or SD up to five days. On day six, unmated males fed on one diet were paired with unmated females fed on either of the remaining two diets, and then the pairs were fed on each of the test diet for 18 days. Total 10 combinations of the diet sequence were made, each of which had three replicates. We found that the YH-deprived females failed to mature sexually. We measured the length and area of the testes and the length, width, and area of the apodeme, and assessed ovarian development. The diet regimes that included YH outperformed those that did not. MD is not a suitable adult diet to replace YH for Q-fly SIT programs.

Key words: *Bactrocera tryoni*, sterile insect technique, protein, reproductive development

Improving the field performance of *Anastrepha fraterculus* sterile males through specific refreshing protocols and pre-release treatments

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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. During the first part of the project, we focused on testing the competitiveness of the newly develop sexing genetic strain of A. fraterculus against wild/wildish populations of different regions of Argentina. To this end, we carried out mating competitiveness test under field cages conditions in which wildish males and females from 4 populations from Argentina were released together with males from the genetic sexing strain of *A. fraterculus* termed BP-AF-89, developed at the IPCL. Wild flies from different populations from Argentina were obtained from guavas collected at: Buenos Aires city, Concordia, Tucuman and Yuto. Pupae from these colonies were used at F3 generation, still considered to resemble wild populations. F3 pupae were shipped to the IPCL in Seibersdorf, where they were separated by sex after emergence and placed in round plexiglass cages with water and food until they were sexuallly mature. Pupae from the GSS were obtained following standard rearing techniques developed at the IPCL. GSS males were used when they were sexually mature (10-12 days). During the test 25 males of the two types (BP-AF-89 and wildish) were released inside large walk-in cages deployed in the greenhouse of the IPCL, together with wildish, virgin, females. With the number of mating obtained by each type of male, we calculated the Relative Sterility Index (RSI). The significance of the RSI was evaluated using a paired t-test, comparing the number of matings obtained by each type of male. Results showed that the there are no differences between males from each of the tested populations and those from the GSS in terms of number of mating attained. For the four populations, RSI was around 0.5 (Buenos Aires city: 0.519; Concordia: 0.438; Tucuman: 0.519; Yuto: 0.476). Paired t-test did not detect significant differences between males in any of the analysed populations ($p > 0.05$). Latency and mating duration times are still under analysis.

Influence of dietary protein on performance of sterile *Bactrocera dorsalis* and *Bactrocera correcta* male

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Abstract

Protein in adult diets plays an essential role in the quality of fruit fly rearing, and particularly in mass fruit fly production for SIT method. Although protein is essential for fruit fly development, some researchers have suggested that a large amount of protein may have detrimental effect on the flies. On the other hand, methyl eugenol is known as an attraction agent for fruit fly male. Hence, the aim of our project is to determine the best protein formulas which could be supplemented with methyl eugenol to find out the solution in enhancing the mating success of sterile fruit fly after being released. Our study has been conducted since 2022 on sterilized flies emerged from pupal which were at the same size of 12 - 12.3 mg and radiated at dose of 90 Gy (*B. dorsalis*) and 80 Gy (*B. correcta*). Sterilized flies were continuously reared in cages in the laboratory at a temperature of 26-28°C, 60-80% RH and photoperiod 10: 14 hours (L: D). Flies were fed with five diet treatments, including (T1) Sugar (Y^-:S^+) ; (T2) Hydrolyzed Yeast Enzymatic: Sugar of proportion is 1:2 ($1Y^+$:$2S^+$); (T3) Hydrolyzed Yeast Enzymatic: Sugar of proportion is 1:4 ($1Y^+$:$4S^+$); (T4) Hydrolyzed Yeast Enzymatic: Sugar of proportion is 1:6 ($1Y^+$:$6S^+$); (T5) Hydrolyzed Yeast Enzymatic: Sugar of proportion is 1:12 ($1Y^+$:$12S^+$). Wild male and female flies were collected from infested fruits (guava, dragon fruit) and separated right after emerging. Results from the first year showed that the T2, T3 (Hydrolyzed Yeast Enzymatic: Sugar with the proportion of 1:2; 1:4) are promising diet, and yeast protein ingredient in the adult diet would contribute to affect to the maturation and mortality rate of sterilized males. In addition, it may not influence to the body size of sterilized males of *B. dorsalis* and *B. correcta* fly.

Optimisation of Queensland fruit fly production and rear out systems

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Abstract

Queensland fruit fly, *Bactrocera tryoni* (Froggatt) and other minor species are among the most significant pests of Australia's $15 billion horticulture industry. The sterile insect technique (SIT) is considered a very effective management tool against this pest. In Australia, the New South Wales Department of Primary Industries has been pioneering the SIT against Queensland fruit fly (Q-fly) for the last 25 years and is one of the major partners in the largest SIT R+D projects in Australia, totalling more than 60M$. Several improvements on sterile insect production and transportation processes have been achieved in an earlier 7-year research program, but implementation of this method is reliant on adoption of the technical advances in an operational setting. Hence, to validate, improve and implement this research knowledge gained on SIT within an operational in-field scenario, we undertook a pilot sterile Q-fly field release program. Flies were reared in the mass Q-fly production facility in Port-Augusta, SA and two million irradiated pupae were transported each week to the field rear-out centres in Yanco, NSW and Tatura, Vic. Flies that emerged from the pupae were fed with sugar, water and yeast hydrolysate and held in the rear-out rooms at 26±1 ºC, 65±5% RH for five days prior to release into the field each week using a light Cessna aircraft. Traps baited with cue lure were established 400m apart in the SIT release (treatment) and non-release (control) sites and trapped flies were collected each week from these traps. Here, we present and discuss the results for the quality control (QC) tests, field release re-capture and their relationships for the rear-out centre at Yanco, NSW. We also investigated the effect of sterile female Q-fly sting on fruits under experimental conditions, and these results will be briefly discussed. Finally, the results on the initial trials undertaken to evaluate the suitability of the Mubarqui diet as a protein supplement to adult Q-fly and *Zeugodacus cucumis* (French) (Cucumber fly) will be discussed.

SESSION III: Field Operations (Surveillance systems and control methods)

Development and optimization of infochemical-derived lures for monitoring *Anastrepha* fruit flies

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ORGANIZATION: El Colegio de la Frontera Sur

Abstract

Relatively, little has been explored about the use of male and host fruit volatiles for trapping *Anastrepha* fruit flies. In this proposal, we aim to develop and optimize lures derived from male and fruit volatiles for monitoring *A. ludens* and *A. obliqua*. Here, we report results of the first two specific objectives. The attraction of *A. ludens* females was affected by blend type and feeding regimen but not by sexual status or interactions. Females were more attracted to white sapote blend than sour orange and guava blends. The difference between white sapote and yellow chapote was not significant. Females fed with sugar were more attracted to blends than those fed with sugar-protein (3:1). Males were also more attracted to white sapote blend than to other fruit blends tested. We found a significant interaction between sexual status and blend type. We also found that a major component of white sapote blend was as attractive to both sexes as the 5-component blend. In contrast, the attraction of *A. obliqua* females was affected by feeding regimen but not by blend type or sexual status. The interaction between the sexual status and feeding regimen was significant. Sexually immature females fed with sugar-protein and sexually mature females fed with sugar only showed the highest responses to fruit lures. A similar situation was observed with males. We found that a blend composed of three major components was as attractive to female flies as the 9-component blend derived from *Spondias mombin* fruits. Males did not show a preference for the 9-component blend or the reduced blends. However, males fed with sugar-protein showed better responses to fruit blends than those fed with sugar. On the other hand, the agreed standard protocol to evaluate new food attractants and formulations for *Anastrepha* fruit flies was implemented during the rainy season in the Soconusco region of Chiapas, Mexico. Seven species of *Anatrepha* flies were captures, *A. obliqua* was the most abundant, followed by *A. ludens* (both wild and sterile), *A. serpentina*, and *A. distincta*. The most effective attractant was Ceratrap compared to Torula, Torula with shrimp powder, and Vial-lure 2C.

Development of fruit fly attractants base on brewery yeast wastes in Burkina Faso

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ORGANIZATION: Institut de l'Environnement et de Recherches Agricoles

Abstract

Fruit flies are pests of economic importance in Burkina Faso. They attack the fruits of around thirty plant species, including the mango tree. Our research work on this group of pests focuses on the development of food/sex attractants using waste yeast from breweries and local plant extracts. To do this, trials were conducted in mango orchards from April to July 2022 to identify a food attractant in view to contribute to control fruit flies in sustainability. The following treatments were tested: pure water, torula yeast, waste yeast without supernatant, waste yeast with supernatant, waste yeast without supernatant diluted to 50% and waste yeast with supernatant diluted to 50%. Each treatment was repeated 3 times. Six McPhail traps were used per treatment in each replicate. The traps were pesticide free and each contained 400 ml of a single treatment. They were observed weekly to collect the captured flies and replace the product. No fruit flies were caught in the traps containing pure water. Other traps using brewer's yeast waste or torula yeast captured a total of 35610 (yeast waste with supernatant) to 92530 (torula yeast) fruit flies, consisting of 10-11 species belonging to the genera *Bactrocera*, *Ceratitis*, *Dacus* and *Zeugodacus*. *Bactrocera dorsalis* was the majority in the catches with proportions of 93.23 to 96.30% in the traps containing yeast waste and 75.10% in the torula traps. Catches consisted of 50.67-55.39% for waste yeast and 57.78% for torula yeast. After 15 weekly trap surveys, torula yeast showed an average daily catch index of 37.36 flies/trap/day while the different yeast waste formulations showed 17.85 (yeast waste with supernatant) to 25.78 flies/trap/day (yeast waste with supernatant diluted at 50%). No significant difference ($c^2 = 5.6916$; df = 4; p-value = 0.2234) was observed between the treatments. Yeast waste with supernatant diluted to 50% presents an interesting potential of attractiveness and could be a technology for the control of fruit flies in Burkina Faso.

Keywords: *Bactrocera dorsalis*, mango, host plant, agroecology management.

Quantifying insect trap network captures using TrapGrid

AUTHOR (S): Nicholas C Manoukis

ORGANIZATION: USDA-ARS Hilo Hawaii USA

Abstract

I present a high-level overview of the goals of trap networks, some examples and details for the case of Tephritid fruit flies, and then a detailed description of the TrapGrid model. TrapGrid can be used to quantify the probability of capturing insects instantaneously or over time using a function that relates distance from a given trap to probability of capture and two models of insect dispersal. I give a brief description of other modeling approaches to these questions, some of which have seen application outside of research. I then describe applications of TrapGrid, including to determine trap attraction (the parameter λ in the model) and on a way to compare alternative trap layouts on a landscape scale. Finally, in the practicum, an example is worked on how to compare two alternative trapping layouts in a 1 km2 area via quantification of capture probability instantaneously and over 30d. There remain other, as yet undescribed, applications of TrapGrid and similar models to improve insect pest monitoring, surveillance, and control. This work can serve as a useful starting point to others interested in these potential applications or in practical problems they face.

Redesigned E-Trap and Development of a Trap Deployment Strategy for Fruit Fly Surveillance

AUTHOR (S): David Nestel, Victor Alchanatis, Ben Shaked, Yafit Cohen and Eitan Goldshtein

ORGANIZATION: Agricultural Research Organization, Israel

Abstract

During the last year, we upgraded and improved the McPhail E-trap developed in the EU project FF-IPM, aimed for surveillance of invasive and expanding fruit flies. Improvements includes the integration of all electronic and optical elements into a single module, expected to reduce electronic problems. In addition, the new E-trap incorporates a GPS system, to automatically locate its position in space, and lateral accesses to the inner lumen of the trap to increment the trapping of fruit flies entering from the side (and not only from the bottom). An additional aspects that was improved is the reduction of energy requirements by synchronizing the daily activation of the trap with the cellular communication. This allowed to reduce the amount of batteries from six to three.

An additional aspect being developed in the FF-IPM project is a strategy to optimize the deployment of traps in the geographic space of area-wide projects. We will present the concepts and some preliminary results. In addition, we will present current aims to develop a tool to automatize decision making of trap deployment.

Strengthen South Australia's fruit fly response program through a model-based adaptive management tool and targeted applied research

AUTHOR (S): Katharina Merkel

ORGANIZATION: SARDI-PIRSA

Abstract

Real-world programs to manage fruit fly pests can be massive and complex with unpredictable events further challenging their success. The aim for our studies is to improve 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.

First trials on evaluating alternative field management tools via laboratory and semi-field studies found that alternative products have the potential to attract and kill flies at comparable rates to established products. We compared three approaches and assessed their suitability to generate powerful data in a short time. Greenhouse trials are underway that incorporate the learnings from the first trials.

A scoping study on model-based adaptive management was finalized and a plan developed to progress the development of an integrated model. Based on workshops and consultation three key questions were identified that should be addressed by this approach:

1) What is the risk of new incursions from different pathways (freight, Passenger, other)?

2) What is the probability of fruit fly presence/ absence at a given location before and after treatment at the location?

3) What is the best allocation of resources between surveillance and treatment controls?

The study further identified critical data and model requirements to address selected questions. A wide range of quantitative models are available to estimate risks of fruit fly entry, establishment and spread, as well as the effectiveness of surveillance and risk mitigation interventions. The team recommended that the models are integrated into the Australian Plant Pest and Disease modelling framework (APPDIS) to maximise the “bang for buck” of fruit fly management.

ANNEX 5

STANDARD FAO/IAEA PROTOCOL FOR TRAP EVALUATION

Validation of Trapping Technologies Against Fruit Fly Pests

Standardized Protocol

FAO/IAEA Food and Agriculture Programme

Objetive

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

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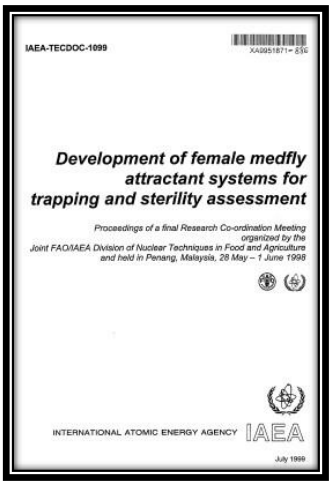
Standard Methodology

1986 – 1992*

1993 - 1998



2000 - 2005

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

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

Trap Types and Attractants

Fruit fly trapping has become highly specialized

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Materials

Bioassays and Fruit Fly Traps and Attractants for Validation:

BIOASSAYS/FF SPECIES	TREATMENTS	BLOCKS/ REPETITIONS	WEEKS	MATERIALS COUNTRY/YR
1 *Anastrepha spp* (Female biased)	A = MLT/Torula Yeast + Shrimp Powder B = Carousel/Vial 2C + PG C = MLT/Ceratrap D = MLT/Torula (Control)	5 Blocks (4 treatments/block) 3 Replicates	8	MLT = Carousel = Torula yeast + shrimp powder = Vial 2C = Ceratrap (lt) = Torula Pellets = Propylen Glycol (LT) =
2 Multiple species (male specific) *Ceratitis capitata, C. rosa, C. cosyra*	A = JT Plastic/TML 2 gm B = JT Plastic/Ceralure 2 gm C= JT Cardboard/TML 2 gm (Control) Note.- JT with inserts 2 g of stickem per insert	5 Blocks (3 treatments/block) 3 Replicates	8	JT Plastic = JT Cardboard = TML 2g = CeraLure 2g = Inserts JT = Stickem

Continues next page....

Materials

3 Multiple species (female biased) *Ceratitis capitata, C. rosa, C. cosyra*	A = MLT/Biolure Unipack (Patch) + PG B = Carousel Trap/Vial-Lure 3C C = Phase IV Plastic/Biolure Unipack D = Phase IV Plastic/Vial-Lure 3C E = MLT/Torula (Control) Note.- Phase IV with inserts 2 g of stickem per insert	5 Blocks (5 treatments/block) 3 Replicates	8	MLT = Carousel Trap = Phase IV Plastic = Biolure Unipack = Vial-Lure 3C = Torula (pellets) = Carousel = PG (Lt) = Inserts FIV = Stickem (kg) =
4 Multiple species (female biased) *Bactocera dorsalis, B. tryoni, B. zonata, Dacus ciliatus, Z. cucurbitae*	A = Carousel Trap/Vial-Lure 3C B = Phase IV Plastic/Vial-Lure 3C C = MLT/Vial 2C + PG D = MLT/Ceratrap E = MLT/Torula (Control) Note.- Phase IV with inserts 2 g of stickem per insert	5 Blocks (5 treatments/block) 3 Replicates	8	MLT = Carousel Trap = Phase IV Plastic = Vial-Lure 3C = Vial 2C = PG (LT) = Ceratrap = Torula (pellets) = Inserts FIV = Stickem (kg) =

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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

✓ For example, five (5) blocks (I to V) each with four (4) treatments (A, B, C, 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 **8** weeks with weekly trap revisions

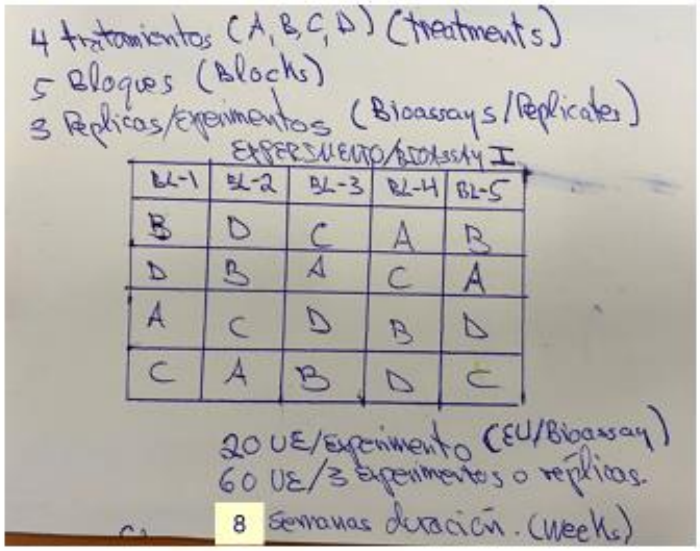
✓ Bioassays should be repeated every year (5 years)

✓ Depending on the type of attractant the replacement of the lures should be established (see slide 9):

- Long lasting para-pheromones and synthetic food lures should not be replaced
- Attractants with short life such as 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
(Example)



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 8 weeks.

Replacement of Trap Devices and Attractants

Attractants Replacement:

- ✓ In bioassay #1, the Torula Yeast + Shrimp Powder, and the Torula (Control) will be replaced every week (8 times). The Ceratrap will be replaced every 4 weeks (2 times) and the Vial-Lure 2C will not be replaced (8 weeks in the field).
- ✓ In bioassay #2, the TML and Ceralure will not be replaced (8 weeks in the field).
- ✓ In bioassay #3, the Biolure Unipack, Vial-Lure 3C will not be replaced (8 weeks in the field), while the Torula will be replaced every week (8 times during the bioassays). The Propylene Glycol solution at 20% will be replaced every 4 weeks (2 times in total).
- ✓ In bioassay #4, Vial-Lure 3C and Vial 2C will not be replaced (8 weeks in the field), the Ceratrap will be replaced every 4 weeks (2 times), while the Torula will be replaced every week (8 times during the bioassays). The Propylene Glycol solution at 20% will be replaced every 4 weeks (2 times in total).

Trap Bodies Replacement:

- ✓ In bioassay #1 the MLT will not be replaced.
- ✓ In bioassay #2, the plastic JT bodies will not be replaced. The cardboard bodies will be replaced every 4 weeks (2x in total) and the JT inserts every week (8 times).
- ✓ In bioassay #3 and #4, the MLT, Carousel Trap and the Phase IV Plastic Traps will not be replaced. Inserts of Phase IV will be replaced every week (8 times).

Trap Preparation

- The volume of solution in MLT traps should be 300 ml when Torula Yeast (TY) is being used as an attractant.
- 4 Torula pellets (5 gr each) should be used per trap. The Torula pellets should be dissolved in the 300 ml water.
- Torula solutions should be replaced every week.
- When Propylene Glycol (PG) is being used as a retention system and to reduce evaporation in the MLT trap baited with the Biolure Unipack and the Vial 2C, a 300 ml solution of water and PG at 20% concentration should be used with.
- The PG solution should be replaced every 4 weeks (two replacements in the 8-week bioassays).
- The volume of Ceratrap in MLT traps should be 500 ml following the products label.
- MLT traps baited with Torula and Ceratrap should be replenished with water when required to reactivate the attractant. A portion of the solution is lost through evaporation. Replenishment with water in the traps should be recorded since it impacts the costs of operating the traps.
- One package of Torula + shrimp powder (equivalent to 5 Torula pellets) should be used per MLT trap.

Methods

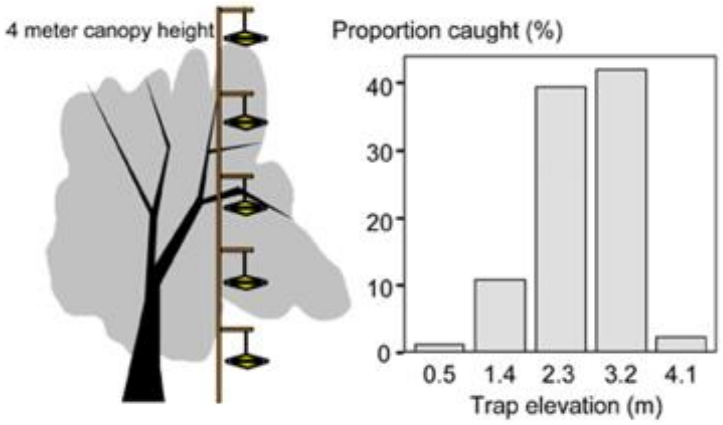
- **Trap placement**

✓ 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

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