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WORKING MATERIAL

IMPROVEMENT OF COLONY MANAGEMENT IN INSECT MASS-REARING FOR SIT APPLICATIONS

THIRD RESEARCH COORDINATION MEETING

ORGANIZED BY THE
JOINT FAO/IAEA DIVISION OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

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1. SUMMARY

Sterile Insect Technique (SIT) applications against major insect pests and disease vectors rely on the cost-effective production of high-quality sterile males. This largely depends on the optimal management of target pest colonies by maximizing the benefits provided by a genetically rich and pathogen-free mother colony, the presence of symbiotic microorganisms, and an efficient domestication and mass-rearing process, while at the same time minimizing or even eliminating the outbreak of microbial (bacteria, fungi, microsporidia) and viral pathogens, as well as the use of hazardous chemicals. The optimization of the colony management for different SIT target insects will ensure a standardized high-quality mass-rearing process and the cost-effective production of sterile males with enhanced field performance and male mating competitiveness. The proposed CRP aims to develop best practices for insect colony management for the cost-effective production of high-quality sterile males for SIT applications against major insect pests and disease vectors through a multidisciplinary approach involving entomologists, geneticists, ecologists, microbiologists, pathologists, virologists, and mass-rearing experts.

2. BACKGROUND SITUATION ANALYSIS

Wild and captive insect populations constantly evolve as a result of both random and adaptive processes. Adaptation is a non-random process by which individuals carrying certain genotypes have a fitness advantage over other individuals, such that the frequency of favored genotypes increases in the population over time. However, an individual's fitness is context-dependent, whereby genotypes conferring high fitness under certain environments may confer low fitness under different conditions. Mass-rearing of insects for SIT poses a particular challenge in this regard, as the insects reared in the production facility face two strikingly different environments during their lifetime: the rearing environment (the facility) vs. conditions in field, where they will be released for the sole purpose of achieving matings with wild females in the presence of competing wild males. In addition, because the targeted reproductive fitness of released males is zero, adaptive processes only operate under the rearing environment. Therefore, unless mitigating efforts are implemented, the field performance of mass-reared sterile males is bound to deteriorate over time. In addition, other diversity-eroding processes may occur: i) a small founding population can restrict genetic diversity from the beginning; and ii) genetic drift resulting from the random loss of genetic variation that is most pronounced in small populations. Parallel phenomena can occur with microorganism communities associated with insects in the mother colony.

In addition to the functions encoded by their own genome, the fitness of multicellular organisms is greatly influenced by the communities of microorganisms that they harbour in or on their bodies. These associated microbial populations can have extensive effects on their hosts, including nutritional and protective benefits. In general, due to their short generation time, larger population sizes, and in some cases, environmental acquisition, microbial communities may confer hosts with a greater capacity to tolerate or resist biotic and abiotic challenges. Microbial community composition and function may itself evolve rapidly with host populations as a result of selective and random processes. An extreme example of this is the loss or replacement of particular microbial partners during domestication of wild insects. Thus, the symbionts that may have beneficial effects on their host in the wild, including traits affecting male mating performance, may be lost during the mass-rearing process. Furthermore, such a shift in the symbiotic community may expose the mass-reared insect to increased sensitivity to pathogens.

The challenges to be met are therefore:

1. To prevent or minimize deterioration of the mother colony by maintaining genetic diversity.
2. To prevent or minimize the loss of field performance.

3. To identify and conserve the symbiotic potential that enables the insect to combat entomopathogens and succeed upon field release.

Effective colony management is essential for insect mass-rearing and the successful application of the sterile insect techniques. We propose to address four major problems encountered during insect colonization and insect mass-rearing:

1- Low genetic diversity, entomopathogen presence, and low performance are regularly encountered during insect colonization and adaptation to mass-rearing conditions.
2- Loss of genetic diversity and important symbiotic organisms often leads to a decline in mating competitiveness, predator avoidance, and longevity, as well as circadian rhythm alteration, resulting in colony deterioration, as observed under continuous mass-rearing.
3- Loss of strain stability or purity of specially designed or selected insect strains are major concerns during continuous mass-rearing, resulting in colony deterioration.
4- Colonies in insect mass-rearing facilities are frequently threatened by infection or build-up of microbial and viral entomopathogens, which is exacerbated by the lack of pathogen detection tools.

1. Low genetic diversity, presence of entomopathogens, and low performance are regularly encountered during insect colonization and adaptation to mass-rearing conditions.

The establishment of new colonies for mass-rearing typically involves collection of wild specimens to breed in captivity. The process of domestication necessarily results in individuals that differ from their wild counterparts. Such differences arise from the result of selection for traits that are beneficial to the mass-rearing process, or genetic drift stemming from small effective population sizes, which are generally lower than census sizes. The size of the founding population, along with other demographic parameters, will influence the degree to which genetic drift will erode genetic variation. Substantial loss of genetic diversity can compromise the ability of the colony and of the released insects to respond to environmental stressors, including evolving pathogens, in both rearing and field conditions. Similarly, small population sizes can lead to a reduction in fitness caused by the exposure of deleterious recessive alleles resulting from inbreeding (i.e., inbreeding depression). In addition, selection for traits favored by the domestication process (e.g. high fecundity and early reproduction) is often negatively correlated with traits associated with field performance (e.g. survival, mating competitiveness, predator avoidance). Changes in the microbial community can also result in reduced performance.

Because the size of a founding population is limited by economical and logistical factors, determination of the minimum founding size and traits required for successful establishment is desirable. Similarly, to monitor genetic and microbiota (including pathogens) changes, it is important to develop adequate methodologies and establish baseline measures.

2. Loss of genetic diversity and important symbiotic organisms often lead to decline in mating competitiveness, predator avoidance, and longevity under stress resulting in colony deterioration, as observed under continuous mass-rearing.

Empirical evidence shows that the insect mass-rearing process over generations normally results in adaptations of the target insect species to facility conditions that allow production of large numbers. Examples of these adaptations are female acceptance of oviposition devices, early reproduction, fast development, and high fecundity rates. For SIT application, this is highly desirable, since it makes the process more cost-effective.

However, it has been observed that there are trade-offs of these adaptations with field performance. One such trait is sexual competitiveness. Under mass-rearing conditions, female mate choice is affected by high densities and the operational sex ratio, resulting in less-selective females. This in turn results in selection for less elaborate male courtship behaviours and the concomitant loss of mating competitiveness.

Another common trait of mass-reared insects is the loss of their ability to avoid predation, which results in very high mortality under natural field conditions. Alteration of the circadian rhythm, caused by different environmental conditions or light: dark cycles, is another undesirable adaptation of mass-reared insects, because it reduces encounters of sterile males and wild females.

This deterioration of sterile insects resulting from continuous mass-rearing reduces the effectiveness of SIT and could jeopardize its successful application. Enhanced colony management practices could represent an alternative to overcome these undesirable effects of mass-rearing. There is evidence that classical genetic breeding methods, such as selection and hybridization, could be used to improve field performance of mass-reared insects for SIT.

Symbiotic organisms provide beneficial functions not encoded in the genome of the host organism, enabling it to live in its natural environment. Upon entering mass-rearing, the original community is partially or totally lost and replaced, with potential deleterious effects in terms of protection against pathogens and of field performance. The loss of symbiotic interactions can be mitigated by: i) reconstituting it by exogenously providing adequate microorganisms, or ii) maintaining symbionts by identifying the causes in the mass-rearing process that lead to its elimination. The causes of the loss may be nutritional (e.g. a diet providing all the needs of the insect; a diet in which the symbionts are outcompeted by other microbes or eliminated by antimicrobial compounds), environmental (e.g. temperature, larval density) or it may be due to accompanying genetic changes.
An important outcome of colony management is data integration. Insect population genetics, production parameters, field performance, and symbiont functional and taxonomic partnerships, as they change during colony establishment and production, should be analyzed in a unified framework. The alteration of genetic structure in the host and symbionts could be linked to production, functional, and performance outcomes.

3. **Loss of stability or purity of specially designed or selected strains are major concerns during continuous mass-rearing, resulting in colony deterioration.**

SIT is more efficient when only males are released. Mechanisms to separate sexes during mass-production have been developed for several tephritids and are in preparation for other insects such as mosquitoes. They are based on the generation of chromosomal translocations whereby a portion of an autosome containing a marker gene (e.g. pupal colour) is translocated to the Y chromosome. Under this scheme, males are heterozygotes whereas females are recessive homozygotes for the marker. Strains carrying such translocations are susceptible to loss of the translocation due to rare recombination events. Accumulation of recombinant individuals, where the sexing mechanism has broken down, reduces the efficiency of production and release. Such phenomena can lead to the closure of production plants, which can have serious consequences on control activities over broad geographical areas, thus enabling the build-up of pest populations. A procedure has been designed to maintain strain stability in tephritids, by which recombinant individuals in the mother colony are removed (i.e., males and females with the incorrect pupal colour). It would be advantageous to transfer and optimize such filtering schemes to maintain stability and purity of colonies regarding these and other desirable characteristics (e.g. longevity, competitive ability, tolerance to stress, predator avoidance, etc.), which could enhance performance of released males. In addition, inversions can be integrated in the strains covering the region of the translocation breakpoint and the selectable marker, as the chromosomal inversions can suppress recombination events.

4. **Colonies in insect mass-rearing facilities are frequently threatened by infection or build-up of microbial and viral entomopathogens, which is exacerbated by the lack of detection tools.**

Insects in SIT mass-rearing facilities are reared at high densities under artificial environmental conditions, in terms of food supply, temperature, light and humidity. These production conditions together with a limited genetic diversity of the reared colony may enhance the outbreak of infectious diseases, hampering colony health and therefore threatening the economic production of highly performing insects. Disease may result in sub-lethal effects, such as reduced fecundity, fertility, longevity, or delayed development, or massive mortality and complete decline of a reared colony. Therefore, early detection, monitoring, and prevention measures are crucial to avoid the outbreak and establishment of infectious diseases in insect mass-rearing. Insects used for SIT mass-rearing facilities belong mainly to Diptera, Lepidoptera, and Coleoptera. They may be susceptible to microbial (bacteria, fungi, microsporidia) and viral entomopathogens. Many of these entomopathogens have been well known for decades, whereas others have been only recently discovered, such as the *Glossina pallidipes* salivary gland hypertrophy virus in tsetse fly SIT rearing facilities.

Bacteria associated with insects can be mutualists, commensals, parasites and/or entomopathogens. Entomopathogenic bacteria are found in different bacterial genera, including *Bacillus, Serratia, Pseudomonas, Micrococcus*, and many others. These pathogens may be highly specific or have a broad host range. They generally infect their hosts through oral routes.

Insect rearing may suffer from entomopathogenic fungi. Fungi generally have a broader host range than bacteria or viruses, and harm insects from different orders. In addition to insect-specific entomopathogenic fungi, saprophytes, such as *Aspergillus* or *Penicillium*, may grow on artificial diets and severely affect colony health. Microsporidia and other protists are unicellular eukaryotic organisms, which are phylogenetically related to fungi. Many microsporidia species are known as pathogens of vertebrates and invertebrates, including insects. They are intra- and intercellular parasites, which are horizontally transmitted by spores or vertically transmitted via eggs. Microsporidia infections in insects often culminate in chronic infections, reducing the fitness, fecundity, and other production parameters of produced insects. They may also entail an increased susceptibility to other pathogens.

Insect viruses are thought to be predominantly transmitted via an oral route, though there are examples of vertical transmission. Virus infection may cause a sudden colony decline or a chronic infection affecting colony fitness. Infections caused by DNA or RNA viruses have been reported as a severe threat for SIT mass-rearing colonies: e.g. baculoviruses in codling moth, hytrosaviruses in tsetse flies, densovirus in mosquitoes, and iflaviruses in medfly. Some of these viruses may remain undetected for a long time in covert (latent or persistent) infections in insect colonies and can become activated (resulting in an overt infection) by unknown endogenous factors. Also, exogenous conditions, such as suboptimal rearing parameters or overcrowding, favour outbreak of covert viruses.

Outbreaks of bacterial and fungal diseases can be controlled by implementing hygiene standards, applying sanitary measures, and by adding antimicrobial compounds to the diet. Fungistatic and bacteriostatic measures based on detergents and toxicants can be used, although they may put insect and human health at risk. Therefore, these measures have to be adapted to each insect species used in the SIT. Development of resistance against antimicrobial compounds needs to be prevented at the same time.

In many cases, viral pathogens cannot be eliminated using hygiene standards or non-harmful chemicals. So far, topical treatment with formalin is used to decontaminate egg surfaces, or to be incorporated in insect diet, to reduce virus infection is used in many SIT rearing facilities. Due to the toxic effects of formalin, the use of other antiviral drugs to reduce and/or eliminate the virus load is desirable. Very few antiviral drugs are available, and new antiviral drugs against viral pathogens found in insect mass-rearing for SIT are needed. As an alternative, the recent selection of codling moth strains highly resistant to *Cydia pomonella* granulovirus (CpGV) in the field may provide new opportunities to secure colony health in regard to baculovirus infection. These resistance mechanisms may serve as a molecular model for other lepidopterans that are targets of the SIT. Breeding of insect colonies with an inherited resistance to pathogens may be highly desirable, but this strategy needs to be assessed, because resistance to pathogens may also result in loss of future pathogen-based control opportunities, if these resistant insects become accidentally established in the environment.

Efficient and specific entomopathogen diagnosis is essential for both establishment and maintenance of an insect colony. Entomopathogen diagnosis is based on host symptoms and culture-dependent approaches, light and electron microscopy, as well as molecular tools based on ELISA or PCR. In many cases, these techniques need to be developed for specific host-pathogen systems. Rapid and cost-effective diagnostic tools based on novel molecular techniques, such as microarrays and next generation sequencing, are desirable for future applications.
It is well understood that insect pathogen control strategies based on chemicals (antimicrobial or antiviral materials, etc.) can increase pressure on the pathogens to evolve resistance, if not wisely applied. Therefore, in an ongoing mass-rearing situation, it is desirable to employ pathogen control strategies that mitigate resistance development. Non-chemical disinfection, a wide array of chemical treatments, or rotating control strategies all appear to be desirable options to develop as a method to improve sustainability in terms of insect pathogen control.

3. REVISED LOGICAL FRAMEWORK

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
Overall Objective: The objective of this CRP is to develop the best practices for insect colony management for improved SIT applications against major insect pests by exploiting existing as well as novel knowledge and tools to address problems associated with mother colony deterioration and strain breakdown, symbionts and pathogens,	n.a.	n.a.	The sterile insect technique will continue to be of relevance for the environmentally-friendly and sustainable management of insect pests and disease vectors FAO and IAEA Member States will continue requesting support in relation to the application of sterile insect technique against major pests of agricultural, livestock and human health importance
Specific Objectives: 1. To develop methods to manage insect colonization issues to overcome problems such as loss of genetic diversity, insect pathogen presence and low performance	1. Revised protocols in participating SIT programmes resulting in more effective management of colonization	1. Records of rearing facilities implementing new or improved colonization protocols	1a-There are genetic, microbial, performance and logistic problems during colonization processes that need to be addressed 1b-Mitigation measures can be developed to address the identified issues related to the colonization processes
2. To develop methods to prevent or minimize colony deterioration in terms of loss of genetic diversity, important symbiotic organisms, mating competitiveness, predator avoidance, longevity and change in circadian rhythm.	Revised mass-rearing protocols in participating SIT programmes resulting in minimal colony deterioration	2. Records of rearing facilities implementing new or improved mass rearing protocols	2a-There are colony deterioration processes during continuous insect mass-rearing that need to be prevented or minimized 2b-Mitigation measures can be developed to address the identified issues related to the colony deterioration
3. To develop methods to avoid or minimize the loss of insect strain stability or purity of specially designed or selected strains	Revised protocols resulting in stable and pure strains at participating SIT programmes	3. Records of rearing facilities implementing new or improved mother colony maintenance protocols	3a-Strain breakdown is a common phenomenon that needs to be addressed

			3b-Mitigation measures can be developed to maintain strain stability
4. To develop methods to avoid or minimize the infection or build-up of microbial and viral pathogens in mass rearing facilities and the emergence of resistance against anti-microbial compounds and to develop pathogen detection tools	Revised protocols that result in effective disease control in mass-reared colonies at participating SIT programmes	4. Records of rearing facilities implementing new or improved disease control protocols	4a-Insect mass rearing systems are at high risk of infectious diseases 4b-Continuous use of anti-microbial compounds may select for resistance 4c-Mitigation measures can be developed to prevent the introduction and/or build-up of microbial or viral infection
Outcomes:			
1.1. Methods to measure genetic diversity in a new insect colony and recommendations concerning founding colony for target SIT species	Recommendations concerning founding colony for SIT mass rearing resulting in colonies of adequate genetic diversity	1.1 Scientific reports and guidelines	1.1. Starting colonies of adequate sizes will minimize the loss of genetic diversity
1.2. Lists of entomopathogens that may impact colonies of target SIT species	1.2. Recommendations on pathogens that should be avoided resulting in pathogen-free colonies	1.2. Scientific reports and guidelines	1.2. Highly detrimental insect pathogens can be identified in the process of colonization
1.3. Minimum performance levels established in terms of mass rearing productivity and field performance (sexual compatibility and competitiveness, survival)	1.3. Guidelines on key parameters related to mass rearing productivity and field performance	1.3. Scientific reports and guidelines	1.3. Minimum performance levels can be preserved during the colonization process
2.1. Methods to monitor changes in genetic diversity in an insect colony	2.1. Guidelines for monitoring genetic diversity	2.1. Scientific reports and guidelines	2.1. Genetic changes during insect mass rearing can be adequately estimated
2.2. Methods to measure and remediate the loss of important symbiotic organisms or their functions	2.2. Guidelines to maintain colonies with important symbiotic organisms	2.2. Scientific reports and guidelines	2.2. Important symbiotic organisms can be maintained or reintroduced into mass-reared colonies
2.3. Methods to measure or mitigate the loss of mating competitiveness, predator	2.3. Recommendations for maintaining colonies with	2.3. Scientific reports and guidelines	2.3. Mitigation measures can be developed to address the identified

avoidance, longevity, or circadian rhythm in target SIT species	adequate field performance		issues related to the colony deterioration
3. Methods to measure and maintain stability and purity of specially designed or selected insect strains	3. Recommendations for maintaining pure and stable colonies	3. Scientific reports and guidelines	3. Strain breakdown is a common phenomenon that can be addressed through mitigation measures
4.1. Availability of diagnostic tools to monitor the prevalence of entomopathogens in insect colonies.	4.1. Guidelines for applying diagnostic tools for pathogen detection	4.1. Scientific reports and guidelines	4.1. Insect pathogens can be detected and quantified by diagnostic tools
4.2. Sanitary measures to avoid the outbreak and spread of insect diseases	4.2. Guidelines for maintaining colonies free of pathogens and diseases	4.2. Scientific reports and guidelines	4.2. Colony health can be improved by sanitary measures
4.3. Disease resistant insect strains for SIT mass rearing	4.3. Recommendation for establishing disease-resistant strains	4.3. Scientific reports and guidelines	4.3. Pathogen-resistant insect strains can be a highly valuable asset to suppress disease outbreaks
4.4. Alternative entomopathogen management strategies	4.4. Recommendations on alternative pathogen management strategies in insect mass rearing	4.4. Scientific reports and guidelines	4.4. Integration of alternative entomopathogen control methods improves colony health
Outputs			
1.1.1. Genetic markers developed for target SIT species	1.1.1. Genetic markers developed for at least four SIT target species	1.1.1. Scientific reports and peer reviewed publications	1.1.1. Adequate genetic markers can identified and optimized
1.1.2. Methods compared to measure genetic diversity in a new insect colony	1.1.2. Three methods compared in at least two SIT target species	1.1.2. Scientific reports and peer reviewed publications	1.1.2. Different approaches to measure genetic diversity are available
1.1.3. Models developed to optimize minimum founding colony size for target SIT species	1.1.3. Model on founding colony size developed using population viability analysis for different SIT target species	1.1.3. Scientific reports and/or peer reviewed publications	1.1.3. Minimum founding colony size can be estimated through modeling
1.2.1. Diagnostic tools for known entomopathogens developed	1.2.1. Diagnostic tools developed for major entomopathogens of at least four target insect species	1.2.1. Scientific reports and peer reviewed publications	1.2.1. Highly detrimental insect pathogens can be identified in the process of colonization

1.2.2. Lists of entomopathogens that might impact colonies of target SIT species developed	1.2.2. List of entomopathogens developed for at least four SIT target species	1.2.2. Scientific reports and/or peer reviewed publications	1.2.2. Highly detrimental insect pathogens can be identified in the process of colonization
1.3.1. Minimum performance levels established in terms of mass rearing productivity	1.3.1. Minimum performance levels established under mass rearing for at least four SIT target species	1.3.1. Scientific reports and/or updated manuals and guidelines	1.3.1. Minimum performance levels can be preserved during the colonization process
1.3.2. Minimum performance levels established in terms of field performance (sexual compatibility and competitiveness, survival)	1.3.2. Minimum field performance levels established for at least four SIT target species	1.3.2. Scientific reports and/or updated manuals and guidelines	1.3.2. Minimum performance levels can be preserved during the colonization process
2.1. Genetic changes in insect colonies under mass rearing monitored	2.1. Genetic changes during continuous mass rearing monitored in at least four SIT target species	2.1. Scientific reports and peer reviewed publications	2.1. Genetic changes during insect mass rearing can be adequately monitored
2.2.1. Diagnostic tools applied or developed for important symbiotic organisms	2.2.1. Diagnostic tools for important symbiotic organisms applied in at least four SIT target species	2.2.1. Scientific reports and peer reviewed publications	2.2.1. There are important symbiotic organisms that can be identified and characterized
2.2.2. Methods developed to assess loss of important symbiotic organisms or their functions during continuous mass rearing	2.2.2. Changes in symbiotic organisms monitored during continuous mass rearing in at least four SIT target species in at least three species	2.2.2. Scientific reports and peer reviewed publications	2.2.2. There are important symbiotic organisms that can be maintained in mass-reared colonies
2.3. Remediation methods to address the loss of essential symbiotic organisms or their functions assessed	2.3. Approaches to promote beneficial microbiota assessed in at least two SIT target species	2.3. Scientific reports and peer reviewed publications	2.3. There are important symbiotic organisms that upon loss can be reintroduced into mass-reared colonies
2.4. Methods to mitigate the loss of mating competitiveness, predator avoidance, longevity or circadian rhythm in target SIT species developed	2.4. Mother colony management methods assessed under low stress conditions to mitigate the loss of mating competitiveness, predator avoidance, longevity or circadian rhythm in	2.4. Scientific reports and peer reviewed publications	2.4. Mitigation measures can be developed to address the identified issues related to the colony deterioration

	at least two target SIT species		
2.5. Investigate potential for cryopreservation to preserve insect colonies	2.5. Protocol developed/assessed to cryopreserve insect colonies in at least two SIT target species	2.5. Scientific reports and/or updated manuals and guidelines	2.5. A protocol for cryopreservation of insects can be established for target SIT species
2.6. Cryopreservation to preserve the insect microbiome	2.6. Cryopreservation of the insect microbiome explored in at least two SIT target species	2.6. Scientific reports and/or updated manuals and guidelines	2.6. A protocol for cryopreservation of insect microbiome can be developed for target SIT species
3.1. Methods assessed to measure and maintain the stability of specially designed or selected insect strains	3.1. Stability of specially designed or selected strains assessed in at least two SIT target species.	3.1. Scientific reports and peer reviewed publications	3.1. Protocols to maintain the stability of specially designed or selected strains can be established
3.2. Best practices developed to avoid colony contamination to maintain colony purity	3.2. Best practices to avoid colony contamination to maintain colony purity developed in at least one SIT target species.	3.2. Scientific reports and peer reviewed publications	3.2. Colony contamination remains an important problem in insect mass rearing Best practices can be achieved to maintain colony purity
4.1. Diagnostic tools developed to monitor the prevalence of entomopathogens in insect colony	4.1. Diagnostic tools for routine monitoring of key entomopathogens developed for at least two SIT target species	4.1. Scientific reports and peer reviewed publications	4.1. Insect mass rearing systems are at high risk of infectious diseases Important pathogens for insect mass rearing can be diagnosed
4.2.1. Endogenous and exogenous factors contributing to the outbreak of latent infection identified	4.2.1. Factors causing outbreak of latent infection identified for at least two SIT target species	4.2.1. Scientific reports and peer reviewed publications	4.2.1. Latent pathogen infections are present in insect mass rearing colonies, and become activated by endogenous and exogenous factors
4.2.2. Sanitary protocols defined and assessed to avoid the spread of insect diseases	4.2.2. Sanitary protocols to suppress the spread of key pathogens defined and assessed for at least two SIT target species	4.2.2. Scientific reports and/or updated manuals and guidelines	4.2.2. Mitigation measures can be developed to prevent the introduction and/or build-up of microbial or viral infection

4.3. Disease-resistant insect strains developed and assessed for SIT mass rearing	4.3. Insect strain resistant to specific pathogens developed and assessed under mass rearing condition for at least one SIT target species	4.3. Scientific reports and peer reviewed publications	4.3. Pathogen-resistant insect strains can be effectively used to minimize disease outbreaks
4.4.1. Symbiont-mediated defence strategy investigated	4.4.1. Symbiont-mediated defence strategy investigated for at least one SIT target species	4.4.1. Scientific reports and peer reviewed publications.	4.4.1. Symbiont-mediated protection may be useful for mitigating disease outbreaks in mass rearing
4.4.2. Entomopathogen management strategies that minimize the use of drugs and chemicals investigated	4.4.2. Entomopathogen management strategies for at least one SIT target species investigated	4.4.2. Scientific reports and peer reviewed publications	4.4.1. An integrated approach for pathogen management can be developed to sustain healthy colonies
ACTIVITIES:			
A. Administrative activities			
1. Hold Consultants Meeting and prepare CRP proposal	Consultant meeting held May 2017	Report of consultant meeting and CRP proposal	Consultant meeting approved
2. CRP proposal submitted to IAEA committee	CRP proposal submitted	Minutes of IAEA Committee	CRP proposal approved by IAEA committee
3. Announce project amongst established geneticists, entomologists, microbiologist, and mass rearing managers to establish CRP	CRP announced, and research contract and agreement proposal submitted, evaluated and forwarded to IAEA committee	Issue contracts and agreements	Sufficient Research proposals submitted for the proposed CRP Contracts and agreements approved by IAEA committee
4. Organize first RCM to plan, coordinate and review proposed research activities (2nd quarter 2018)	First RCM held in mid-2018	First RCM report	RCM is funded and approved
5. Carry out R&D as agreed in the first RCM as indicated in R&D activities section	Research carried out by contract and agreement holders	Research reports	Reports approved and subsequent funding of contracts
6. Second RCM to analyse data and develop research plans for the next phase of the CRP (early 2020)	Second RCM held in first half of 2020	Second RCM report	RCM is funded and approved
7. In conjunction with second RCM, and Colony Management"	Workshop held in conjunction with 2nd RCM	Workshop report	Workshop approved
8. Continue R&D as agreed in the second RCM as indicated in R&D activities section	Research carried out by contract and agreement holders	Research reports	Reports approved and subsequent funding of contracts

9. Review the CRP during its third year	Mid-CRP report prepared and submitted to IAEA committee	Mid-CRP report	IAEA committee approves funding for second half of CRP
10. Convene third RCM to evaluate results and plan final research of the CRP (first semester of 2023)	Third RCM held virtually in first quarter of 2023	Third RCM report	RCM is funded and approved
11. In conjunction with third RCM, hold workshop on "Genetic Diversity Analysis and Colony Management "	Workshop held virtually in conjunction with 3rd RCM	Workshop report	Workshop approved
12. Continue R&D as agreed in the third RCM as indicated in R&D activities section	Research carried out by contract and agreement holders	Research reports	Reports approved
13. Hold final RCM to review data and reach consensus (early 2024)	Fourth RCM held in first quarter of 202 4	Fourth RCM report	RCM is funded and approved
14. Evaluate the CRP and submit evaluation report	Final CRP evaluation carried out and submitted to IAEA committee	CRP Evaluation Report	IAEA committee approves final CRP evaluation report
15. Prepare articles for joint final publication of CRP results in a Special Issue of an open source and peer-reviewed scientific journal	CRP participants prepare and submit papers on their research	Special Issue in scientific journal	Special Issue funded Manuscripts submitted survive the peer-review process
B. R&D activities			
1.1.1. Develop genetic markers for target SIT species	Genetic markers and other markers developed for at least four SIT target species	1.1.1. Scientific reports and peer reviewed publications	1.1.1. Adequate markers can be identified and optimized
1.1.2. Compare methods to measure genetic diversity in a new insect colony	1.1.2. Three methods (e.g. RAPD, micro satellites , SNPs) compared in at least two SIT target species	1.1.2. Scientific reports and peer reviewed publications	1.1.2. Different approaches to measure genetic diversity are available
1.1.3. Develop models to optimize minimum founding colony size for target SIT species	1.1.3. Model on founding colony size developed using population diversity analysis for different SIT target species	1.1.3. Scientific reports and/or peer reviewed publications	1.1.3. Minimum founding colony size can be estimated through modeling
1.2.1. Develop diagnostic tools for known entomopathogens	1.2.1. Diagnostic tools (e.g. PCR, qPCR, HTS, FISH) developed for major entomopathogens of	1.2.1. Scientific reports and peer reviewed publications	1.2.1. Highly detrimental insect pathogens can be identified in the process of colonization

	at least four SIT target species		
1.2.2. Develop lists of entomopathogens that might impact colonies of target SIT species	1.2.2. List of entomopathogens developed for at least four SIT target species	1.2.2. Scientific reports and/or peer reviewed publications	1.2.2. Highly detrimental insect pathogens can be identified in the process of colonization
1.3.1. Establish minimum performance levels in terms of mass rearing productivity	1.3.1. Minimum performance levels established under mass rearing for at least four SIT target species	1.3.1. Scientific reports and/or updated manuals and guidelines	1.3.1. Minimum performance levels can be preserved during the colonization process
1.3.2. Establish minimum performance levels in terms of field performance (sexual compatibility and competitiveness, survival)	1.3.2. Minimum field performance levels established for at least four SIT target species	1.3.2. Scientific reports and/or updated manuals and guidelines	1.3.2. Minimum performance levels can be preserved during the colonization process
2.1. Monitor genetic changes in insect colonies under mass rearing	2.1. Genetic changes during continuous mass rearing monitored in at least four SIT target species	2.1. Scientific reports and peer reviewed publications	2.1. Genetic changes during insect mass rearing can be adequately monitored
2.2.1. Develop and apply diagnostic tools for important symbiotic organisms	2.2.1. Diagnostic tools for important symbiotic organisms applied in at least four SIT target species	2.2.1. Scientific reports and peer reviewed publications	2.2.1. There are important symbiotic organisms that can be identified and characterized
2.2.2. Develop methods to assess loss of important symbiotic organisms and their functions during continuous mass rearing	2.2.2. Changes in symbiotic organisms monitored during continuous mass rearing in at least four SIT target species	2.2.2. Scientific reports and peer reviewed publications	2.2.2. There are important symbiotic organisms that can be maintained in mass-reared colonies
2.3. Assess remediation methods to address the loss of essential symbiotic organisms or their functions	2.3. Approaches to promote beneficial microbiota assessed in at least two SIT target species	2.3. Scientific reports and peer reviewed publications	2.3. There are important symbiotic organisms that upon loss can be reintroduced into mass-reared colonies
2.4. Develop methods to mitigate the loss of mating competitiveness, predator avoidance, longevity or circadian rhythm in target SIT species	2.4. Mother colony management methods assessed under low stress conditions to mitigate the loss of mating competitiveness, predator avoidance, longevity or	2.4. Scientific reports and peer reviewed publications	2.4. Mitigation measures can be developed to address the identified issues related to the colony deterioration

	circadian rhythm in at least two target SIT species		
2.5. Optimize cryopreservation methods to preserve insect colonies	2.5. Protocol developed/assessed to cryopreserve insect colonies in at least two SIT target species	2.5. Scientific reports and/or updated manuals and guidelines	2.5. A protocol for cryopreservation of insects can be established for target SIT species
2.6. Optimize cryopreservation methods to preserve the insect microbiome	2.6. Cryopreservation of the insect microbiome explored in at least two SIT target species	2.6. Scientific reports and/or updated manuals and guidelines	2.6. A protocol for cryopreservation of insect microbiome can be developed for target SIT species
3.1. Assess methods to measure and maintain the stability of specially designed or selected insect strains	3.1. Stability of specially designed or selected strains assessed in at least two SIT target species.	3.1. Scientific reports and peer reviewed publications	3.1. Protocols to maintain the stability of specially designed or selected strains can be established
3.2. Develop best practices to avoid colony contamination to maintain colony purity	3.2. Best practices to avoid colony contamination to maintain colony purity developed in at least one SIT target species.	3.2. Scientific reports and peer reviewed publications	3.2. Colony contamination remains an important problem in insect mass rearing Best practices can be achieved to maintain colony purity
4.1. Develop diagnostic tools to monitor the prevalence of entomopathogens in insect colony	4.1. Diagnostic tools for routine monitoring of key entomopathogens developed for at least two SIT target species	4.1. Scientific reports and peer reviewed publications	4.1. Insect mass rearing systems are at high risk of infectious diseases Important pathogens for insect mass rearing can be diagnosed
4.2.1. Identify endogenous and exogenous factors contributing to the outbreak of latent infection	4.2.1. Factors causing outbreak of latent infection identified for at least two SIT target species	4.2.1. Scientific reports and peer reviewed publications	4.2.1. Latent pathogen infections are present in insect mass rearing colonies, and become activated by endogenous and exogenous factors
4.2.2. Define and assess sanitary protocols used to avoid the spread of insect diseases	4.2.2. Sanitary protocols to suppress the spread of key entomopathogens defined and assessed for at least two SIT target species	4.2.2. Scientific reports and/or updated manuals and guidelines	4.2.2. Mitigation measures can be developed to prevent the introduction and/or build-up of microbial or viral infection

4.3. Develop and assess disease-resistant insect strains for SIT mass rearing	4.3. Insect strain resistant to specific entomopathogens developed and assessed for at least one SIT target species	4.3. Scientific reports and peer reviewed publications	4.3. Pathogen-resistant insect strains can be effectively used to minimize disease outbreaks
4.4.1. Investigate symbiont-mediated defence strategy	4.4.1. Symbiont-mediated defence strategy developed for at least one SIT target species	4.4.1. Scientific reports and peer reviewed publications.	4.4.1. Symbiont-mediated protection may be useful for mitigating disease outbreaks in mass rearing
4.4.2. Investigate entomopathogen management strategies that minimize the use of drugs and chemicals	4.4.2. Entomopathogen management strategies developed for at least one SIT target species	4.4.2. Scientific reports and peer reviewed publications	4.4.1. An integrated approach for pathogen management can be developed to sustain healthy colonies

4. INDIVIDUAL WORK PLANS FOR THE NEXT 18 MONTHS

Activities / Names	J. Jehle	O. Manangwa	D.A. Theilmann	M. Ogliastro	Vera Ros	S. Herrero, r	F. M. Khamis	Anne Geiger	F. Njiokou	B. Weiss	S. Pagabeleguem	G. Tsiamis	P. Crisp	D. Haymer	E. Schuenzel	B. Yuval	T. Mastrangelo	A. Bamaca	Ó. Dembilio	P. Liedo	D. F. Segura	R.J.G. Pauza	IPCL
Develop genetic markers for target SIT species (1.1.1)											x			x				x	x				x
Compare methods used to measure genetic diversity in a new insect colony (1.1.2)									x		x			x			x			x			x
Develop methods to optimize minimum founding colony size for target SIT species (1.1.3)									x					x									x
Develop diagnostic tools for known entomopathogens (1.2.1)	x		x	x	x	x							x		x			x					x
Develop lists of entomopathogens that might impact new colonies of target SIT species developed (1.2.2)	x		x	x		x							x		x			x					x
Establish minimum performance levels in terms of mass rearing productivity (1.3.1)						x											x	x	x	x			
Establish minimum performance levels in terms of field performance (sexual compatibility and competitiveness, survival) (1.3.2)											x						x	x	x	x			
Monitor genetic changes in insect colonies under mass rearing (2.1)									x		x			x			x	x	x	x	x		x
Develop and apply diagnostic tools for important symbiotic organisms (2.2.1)							x		x	x	x	x	x		x				x	x	x		x
Develop methods to assess loss of important symbiotic organisms or their functions during continuous mass rearing (2.2.2)		x						x	x	x	x	x	x		x	x			x		x		
Assess remediation methods to address the loss of essential symbiotic organisms or their functions (2.3)										x						x			x		x		x
Develop methods to mitigate the loss of mating competitiveness, predator avoidance, longevity or circadian rhythm in target SIT species (2.4)								x		x				x				x	x	x			
Optimize cryopreservation methods to preserve insect colonies (2.5)													x						x	x			x
Optimize cryopreservation methods to preserve the insect microbiome (2.6)		x										x	x										
Assess methods used to measure and maintain the stability of specially designed or selected insect strains (3.1)																	x	x	x	x			
Develop best practices to avoid colony contamination (3.2)																	x						x
Develop diagnostic tools to monitor the prevalence of entomopathogens in insect colonies (4.1)	x		x	x	x	x	x				x	x	x		x								x
Identify endogenous and exogenous factors contributing to the outbreak of latent infection (4.2.1)	x		x	x	x	x		x															x
Define and assess sanitary protocols defined and assessed to avoid the spread of insect diseases (4.2.2)	x			x									x		x								x
Develop and assess disease-resistant insect strains for SIT mass rearing (4.3)	x		x							x		x											x
Develop symbiont-mediated defence strategies (4.4.1)								x		x	x												x
Develop entomopathogen management strategies that minimize the use of drugs and chemicals (4.4.2)		x		x		x	x				x		x		x								x

5. AGENDA

THIRD RESEARCH CO-ORDINATION Meeting

JOINT FAO/IAEA DIVISION OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

"Improvement of Colony Management in Insect Mass-rearing for SIT Applications"

Vienna, Austria (Virtual)
27th February –3rd March 2023

Monday, 27 February 2023

SESSION 1:

12.00 - 12. 15	**Welcome and introduction**

SESSION 2: Pathogens (Chairperson: Johannes Jehle)

12.15 - 12.35	**Johannes Jehle**, Eva Fritsch, Karin Undof-Spahn, Shili Yang, Fang Shiang Lim, Jörg T. Wennmann: *Cydia pomonella* mass rearing: The contribution of disease resistant codling moth strains for improving colony management.
12.35 - 12.55	**Peter Crisp,** Mohammed Sabbir Sidiqui, Lakshmi Nacey1, David Haymer: Cytogenesis of Bactrocera tryoni update and Lucilia cuprina rearing.
12.55 - 13.15	**Mylène Ogliastro**: Viral metagenomics for large scale sanitory control of insect mass rearing dedicated to SIT.
13.15 - 13.35	**Luis Hernandez-Pelegrin**, Angel Llopis-Gimenez, Cristina Crava, Vera Ros, and **Salvador Herrero**: Covert viral infections in mass-reared medflies: effects, transmission, and evaluation of eradication strategies.
13:35 - 13:55	**A. Mattia,** A. Bryon, **Vera Ros:** Deeper understanding of insects covert viral infections using *Spodoptera exigua* as a model organism.
13.55 - 14.15	**Hannah Huditz**: Protection or harm? New RNA viruses infecting *Glossina morsitans morsitans*.

14.15 - 14.30 **Break**

SESSION 2: Tsetse (Chairperson: Brian Weiss)

14.30-14.50 Asimakis, E., Stathopoulou, P. Bel Mokhtar N., Gouvi, G., Augustinos, A., Caceres, C., Abd-Alla, A.M.M., Bourtzis, K., **Tsiamis, G.:** New insights to the insect bacteriome and virome.

14.50-15.10 Fidelis L.O. Ombura, Adly MM Abd-alla, Steven Runo, Paul O. Mireji, Rosemary Bateta, Inusa J. Ajene, Komivi S Akutse, Joseck O. Esikuri and **Fathiya M Khamis**: Dual suppression of tsetse fly populations using entomopathogenic fungal-based biopesticides and sterile insect technique

15.10 - 15.30 **Flobert Njiokou,** Feudjio Sofack S., Kame Ngasse G. Farikou Oumarou Melachio Tanekou T. and Anne Geiger: Genetic diversity and symbiont specific diversity in wild versus reared populations of *Glossina tachinoides*: estimation of temporal changes.

15.30 -15.50 **Brian Weiss**: Infection with endosymbiotic Spiroplasma disrupts tsetse metabolic and reproductive homeostasis.

15.50 – 16.10 Giulia Fiorenza1, Marco Peviani1, Mauro G. Spatafora1, Andrea Gazzano, Giulia Mancini, Stefano Liberi, Federico Forneris, Irene Rossi, Adly M. M. Abd-Alla, Ludvik M. Gomulski, Giuliano Gasperi, Serap Aksoy, Francesco Lescai1, Geoffrey Attardo and **Malacrida AR:** Reproductive biology of *Glossina morsitans* and *G. fusci*pes.

16.10 – 16.30 **Kiswend-sida M. Dera:** *Spiroplasma* infection in tsetse flies

16.;0 – 16.50 Irene Rossi, Francesca Scolari, Davide Carraretto, Adly M.M. Abd-Alla, Serap Aksoy, Anna Malacrida1, **Geoffrey Attardo**: Defining the Postmating Response in Tsetse Flies: A Systems-Based Analysis of Mating Induced Changes in Morphology, Biochemistry, and Gene Expression

Tuesday, 28st February, 2023

SESSION 2: Tsetse (Chairperson: Brian Weiss) (Continued)

12.00-12.20 **Deusdedit Malulu**, Mustafa Halfani, Athumani Mkinga, Ezra Jana, Peter Paul Lucas and Oliva Manangwa: Studies on the adaptation and performance of wild *Glossina pallidipes* from the coastal areas of Tanzania.

12.20-12.40 Kiswend-sida M. Dera, **Soumaïla Pagabeleguem**, David Haymer, Adly M.M. Abd-Alla: Genetic characterization of four different insectary colonies of *G. palpalis gambiensis*?

12.40 – 13.00 **GSTÖTTENMAYER, Fabian**: Development and characterization of novel microsatellite markers for the tsetse species *Glossina brevipalpis* to study population genetics of laboratory colony and field populations

SESSION 3 Fruit flies (Chairperson: Pablo Liedo)

13.00-13.20 **Carlos Caceres**: The new GSS of Medfly developed at IPCL is a step forward to improving the quality and fitness of sterile males for SIT application.

13.20-13.40 **Erin Schuenzel**, Don Vacek, Bacilio Salas, Hugh Conway, Norman Barr, Roxanne Farris: Microbiome Analysis of *Anastrepha ludens* in lab-reared colonies.

13.40-14.00 Polpass Arul Jose, Boaz Yuval**, Edouard Jurkevitch**: Maternal and host effects mediate the adaptive expansion and contraction of the microbiome during ontogeny in a holometabolous, polyphagous insect.

14.00-14.20 Break

14.20-14.40 **Thiago Mastrangelo**: Current status of colony management for *Anastrepha fraterculus* in Brazil, progress with GSS-89 adaptation, and mating compatibility among populations.

14.40-15.00 **Aparicio Bamaca**, Edwin Ramírez: Handling procedures during colonization and rearing of tephritid fruti flies. maintaining the performance of sterile males of *Ceratitis capitata* wied and *Anastrepha ludens* loew.

15.00-15.20 Jaime García de Oteyza and **Óscar Dembilio**: Dynamics of the Gut Bacteriome during a laboratory adaptation process of the Mediterranean Fruit Fly, *Ceratitis capitata*

15.20-15.40 **Pablo Liedo**, José Salvador Meza-Hernández, Mayren Sánchez Rosario: Mass-rearing colony management for SIT application in Anastrepha fruit flies.

15.40-16.00	Ana Laura Nussenbaum, Micaela Garbalena, Julieta Salgueiro, C. Yañez Prieto, M. Teresa Vera, M. Inés Marchesini, George Tsiamis, Silvia B. Lanzavecchia & **Diego F. Segura:** Gut bacteriome of *Anastrepha fraterculus* sp. 1: characterization and potential use as tools to increase the SIT efficiency.
16.00 – 16.20	Jose Arredondo Gordillo and **Roberto José Gómez Pauza**: LA CABIM-3 FIRST TRIALS
16.20-16.40	**David Haymer**: Use of RAPD markers to monitor the genetic makeup of mass reared colonies of insects.
SESSION 4	**General discussion and forming the working groups (Chairperson; Adly)**
16.20-16.40	**General Discussion and Formation of three Working Groups (see below)** Working Group Discussions, planning and coordinating work programmes

Wednesday 1st March, 2023

12.00 -	**Session 5: General Discussion (Continued)** Working Group Discussions, planning and coordinating work programmes (continued)

Thursday 2nd March, 2023

12.00 -	**Session 6: Working Group Discussions (Continued)** Working Group Discussions, planning and coordinating work programmes (continued) Drafting working group reports and drafting RCM report, Discuss the proposal for the journal for the special issue

Friday 3rd March, 2023

12.00 -	**Session 7: Compiling RCM report** Finalize the RCM report. Discuss the journal and the contribution paper for the special issue **General discussion** Announce 4th RCM place and date and the journal name for the special issue

Closing

Suggestion of group division and chairpersons

Working Group 1: Fruit fly colony management

Pablo Liedo, Diego F. Segura, Óscar Dembilio, Aparicio Bamaca, Thiago Mastrangelo, Edouard Jurkevitch, David Haymer,

Working Group 2: Tsetse fly colony management

Brian Weiss, Soumaila Pagabeleguem, Imna Malele, Anne Geiger, Flobert Njiokou, Fathiya M. Khamis, Anna Malacrida, Geoffrey Attardo

Working Group 3: Pathogens*

Johannes Jehle, David A. Theilmann, Mylène Ogliastro, Salvador Herrero, Vera Ros, George Tsiamis, Erin Schuenzel, Peter Crisp

*Members of this group can interact and participate in the meeting of other groups (Observers can join the discussion group related to their interest).

6. LIST OF PARTICIPANTS

LIST OF PARTICIPANTS TO THE FIRST RCM
ON IMPROVEMENT OF COLONY MANAGEMENT IN INSECT MASS-REARING FOR SIT APPLICATIONS
From 27th February to 3rd March 2023
On line

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*Participant did not attend the 3rd RCM

7. NEXT MEETING

Location: Vienna Or Patras

Period: 4-8 March 2024

Hybrid meeting recommended (in person and on-line)

8. SPECIAL ISSUE

Available proposal:

1- Insects (Special Issue "Advances in Insect Diet and Rearing Methodology (Volume II). https://www.mdpi.com/journal/insects/special_issues/insect_diet_rearing, *Extended to May 1*. Óscar Dembilio

2- Entomologia experimentalis applicata (IF 2.4) (Diago)

3- Journal of Economic Entomology (IF 2.4), special collection ESA (Brian)

4- Journal of Insect Science (IF 2) ESA Adly

5-

6-

7- Symbiosis (IF 3) (not all paper are on symbiosis

8- Frontiers in Insect Science https://www.frontiersin.org/journals/insect-science/about, Pablo Liedo. (No Impact factor)

9- Current Research in Insect Science. (No Impact factor)

10- Journal of Pest Science https://www.springer.com/journal/10340 (IF: 5.742) cost 5100 US$

11- Journal of Integrated Pest Management ESA (IF 4.1) open access 1066 US$