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Improve the Mass–rearing of Lepidoptera Pests for SIT Programmes

Report of the First Research Coordination Meeting of an FAO/IAEA Coordinated Research Project, held in Vienna, Austria, from 5 to 9 September 2022

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Contents

SUMMARY

Lepidoptera are key pests that require control measures to avoid significant losses in many cropping systems worldwide. Failure to control key species can result in significant losses that impact the economics of production. Chemical, semiochemical, and biological measures have been successfully utilized in the control of key lepidopteran pests. However, there have been documented reports of resistance development, deleterious impacts on non-target organisms and high cost. With a worldwide focus on sustainability, there is increasing concern around chemical residues on food and the environmental impact of some of these control measures.

The sterile insect technique (SIT), which involves mass-rearing and releasing sterile insects to overflood a wild pest population, has been used successfully against several moth species. However, despite successes, the wider development and deployment of SIT has important issues that need further investigation. Previous coordinated research projects (CRP) have addressed a number of constraints. Despite excellent progress by a previous CRP, there remains a need for progress in several practical areas that limit the expansion of SIT for Lepidoptera. Having an efficient, cost-effective rearing methodology is the backbone of any successful SIT programme. The processes of mass-rearing that require a research focus include: the development and refinement of working larval diet(s), the development of protocols for pathogen monitoring and management, identification of methods for marking mass-reared sterile Lepidoptera, the development and optimization of egg collection, processing, sanitation, incubation and diet seeding methods and technologies, diet enhancement through additives such as sterols and cryoprotectants and increased automation to improve productivity and reduce costs.

A CRP is proposed to enable collaborators in Member States to benefit from the expansion of the SIT concept to lepidopteran species of economic importance. This objective will be met by focusing on three areas of mass-rearing for SIT, firstly establishing basic rearing technologies for Lepidoptera of economic concern, secondly focusing on scaling, and adapting basic rearing technologies to mass-rearing systems for the application of the SIT against lepidopteran pests, and lastly optimizing existing mass-rearing systems for sterile Lepidoptera to increase efficacy and reduce the cost of existing AW-SIT programmes.

BACKGROUND

Lepidoptera are key pests that require control to avoid significant losses in many cropping systems worldwide. Failure to control key species can result in crop losses and jeopardise the economics of production. Many lepidopteran pests are undergoing geographical range expansion.

According to the current records in the Global Eradication and Response Database (GERDA, http://b3.net.nz/gerda), there are 151 incursion response and eradication programmes around world targeting 32 Lepidoptera species in 15 families. *Tuta absoluta* (tomato leafminer) is a key and expanding threat to tomato production in the Mediterranean, African and Caribbean regions. *Helicoverpa armigera* (old world bollworm) has recently been detected in South American and Caribbean countries. *Lobesia botrana* (European grapevine moth) has been detected in the USA and Chile, and affects grapevine production in many European countries. *Epiphyas postvittana* (light brown apple moth) has recently spread in California, while *Cactoblastis cactorum* (cactus moth) is spreading in the southern USA towards Mexico, where it threatens endemic and valued cacti. *Chilo sacchariphagus* (spotted sugarcane borer) is in danger of spreading south, north and westwards along the eastern coast of Africa, from its confirmed presence in Mozambique, and is present in the Indian Ocean islands of Mauritius, Madagascar and Reunion. In other cases, there is potential to reduce the importance of established pests such as *Cydia pomonella* (codling moth) in Canada, Europe, New Zealand, South Africa, South America, and the USA, which is now also present in north western China. African sugarcane stalk borer *Eldana saccharina* remains very difficult to control in South Africa, Uganda and Zimbabwe. The false codling moth *Thaumatotibia leucotreta* (false codling moth) is a key pest of citrus, stone fruit, and other crops in many countries throughout continental Africa.

Conopomorpha cramerella (cocoa pod borer) is a fairly recent and spreading pest in southeast Asia and the Indo-pacific islands and is considered a good target for SIT. This is also the case for the tortricid, *Thaumatotibia batrachopa* (macadamia nut borer), which is a pest of increasing concern to macadamia farmers in southern Africa.

Control options exist for these pests, but most have issues of cost or efficacy. For example, while insecticides are widely used, there is increasing opposition to non-target impacts. Furthermore, insecticide resistance has been recorded in many of these pests. Control using sex pheromones for mating disruption can be effective under some situations but is comparatively expensive. Classical biological control has also been widely investigated for many of these pests but is usually inadequate alone. The arrival of new invasive species can disrupt biological control, when the new pests require control using insecticides to avoid crop failure. Biopesticides such as *Bacillus thuringiensis kurstaki* and *baculoviruses* also exist for some pests, although cost, efficacy, and resistance development can be problematic. Cultural controls such as sanitation also have to be used as part of integrated pest management, although these tactics are seldom adequate when used alone. The SIT, which involves mass-rearing and release of sterile insects to overflood a wild population of the pest, has been used successfully against many moth species. This technique is more widely developed against fruit flies, in particular, where many countries have successful programs. In several cases the integration of SIT with other tactics has enabled suppression and eradication of moth pests. The best example of this approach is the large area now free of *Pectinophora gossypiella* (pink bollworm) in southwestern USA and northern Mexico, after the combination of *Bt* cotton, sterile insect release and mating disruption. Other examples include the eradication of outbreaks of *Teia anortoides* (Australian painted apple moth) in New Zealand and *C. cactorum* in two Mexican islands. The ongoing area-wide suppression of codling moth in British Columbian apple orchards has also used SIT along with other tactics, and this approach is successfully being applied in South African citrus orchards against *T. leucotreta*.

However, despite these successes, the wider development and deployment of SIT has important issues that need further investigation. Some efforts are currently under development on lepidopteran species such as *L. botrana* in Chile and Argentina, *E. saccharina* in South Africa and *Amyelois transitella* (navel orangeworm) in the Unites States. In addition, previous Coordinated Research Projects (CRP) have addressed a number of constraints. A previous CRP on Lepidoptera SIT (2002-2007) focused on improvements of codling moth SIT to facilitate its field expansion, since this pest has been one of the leading exemplars. More recently, a CRP on Lepidoptera focused on the assessment of moth quality to support the release of factory-reared insects for a wider range of species. It was identified that further expansion of SIT to target other key lepidopteran pests will require improvements in a number of areas. Another recent CRP determined that an increase in the quality control and management of mass-rearing, irradiation, shipping, release and field assessment activities will help to support the development of new programs and increase the efficiency and effectiveness of ongoing programmes. The previous CRP focused on field performance of sterile male Lepidoptera to ensure success in SIT programmes and identified further research and development requirements to enhance the mass-rearing of Lepidoptera. Mass-rearing is only currently available on a few Lepidoptera species, for example *P. gossypiella*, *T. leucotreta* and *C. pomonella*. The ongoing efforts on *L. botrana*, *A. transitella* and *E. saccharina* SIT programmes require the scaling up of mass-rearing systems from experimental rearing systems. Many potential SIT targeted lepidopteran species, such as *C. sacchariphagus*, *T. batrachopa* and *C. cramerella* will require the development of basic rearing technology and systems.

Successful mass-rearing of lepidopteran species for an SIT program requires a comprehensive approach that simultaneously addresses many factors and obstacles. Areas for evaluation and development include the basic rearing technology for candidates of SIT lepidopteran pests, development of working larval diets, protocols for pathogen monitoring and management, mass-rearing systems for pilot-field and commercial-scale production, methods for marking mass-reared sterile Lepidoptera, handling protocols and packing materials for mass-reared Lepidoptera, technology for mechanically separating mass-reared male and female moths (particularly for those species with female-choice mating systems), optimization of egg collection, processing, sanitation, incubation, and diet seeding methods and technology, forecasting and economic analysis of mass-rearing systems for the application of AW-SIT, refining and optimizing larval diets for the more efficient and effective application of existing area wide

SIT programs, increasing the automation of mass-rearing processes, and developing procedures and technology to incorporate diet enhancing additives.

COORDINATED RESEARCH PROJECT (CRP)

This Coordinated Research Project (CRP) is based on a Consultancy Meeting that was held from 8-12 November 2021 in Vienna, Austria (report available) to assess the potential for conducting coordinated R&D in the rearing of Lepidoptera for SIT application, and to formulate a proposal for a CRP on *Improve the Mass-rearing of Lepidoptera Pests for SIT Programmes*.

The overall objective of this new **CRP D4.10.28**, approved for the **period 2022-2027**, is to expand and improve the sterile insect technique for use against lepidopteran pests of crops to improve food security and enhance health and well-being. It focuses on three areas of mass-rearing for Lepidoptera SIT: (1) develop basic rearing for species that have potential for SIT but with no artificial rearing established, (2) scale-up rearing technology for species where rearing is a low scale and (3) improve mass rearing for species under operational programmes

FIRST RESEARCH COORDINATION MEETING (RCM)

Twenty-three scientists from 14 countries attended this first RCM, held in Vienna, Austria from 5-9 September 2022. The list of participants, which included CRP contract and agreement holders, as well as four additional observers, is given in **Annex 1**. The agenda for the meeting is attached in **Annex 2**.

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

During the last three days of the meeting, general discussions were held to define and review the thematic areas of the CRP, the review of the general and specific R&D objectives to be addressed during the 5 years of the CRP, and the CRP Logical Framework, in order to agree on minimum outputs to be achieved at the end of the CRP. Furthermore, participants were divided into two working groups (**Annex 3**) to develop more detailed R&D plans to be conducted during the first 18 months of the CRP.

Abstracts of the presentations are compiled in **Annex 4** and a copy of all presentations was made available to all participants at the end of the RCM.

Mr. Des Conlong from South Africa was invited to have a presentation on “The Comparative Slaughter Technique for Insect Diet Development”. The participants discussed the possibility to use this technique on the development of insect diet. It was agreed that the workshop on carcass milling technique that was proposed in conjunction with the second RCM will be replaced by a diet and host chemical analyses organized by IAEA. All participants identified three main hosts (including the plant part(s) and crop stage) of their working species (**Annex 5**). IAEA will identify a laboratory/company for Proximate and Amino Acid Analyses.

SITUATION ANALYSIS AND RESEARCH THEMES

1. Develop Basic Rearing Technology and Systems for Lepidopteran Pests of Concern

1.1. Develop basic rearing technology for candidate pests

Successful mass-rearing of a pest on its natural host plant has many challenges. These include the host plants seasonal availability, overall excessive costs to grow them in large enough quantities needed for mass-production of insects, and variable insect quality of individuals feeding on the plants (Alfazairy *et al.,* 2012). The advantages of rearing insects under laboratory conditions on controlled scientifically derived diets is an increase in output and the consistency of insect performance. Assuming good nutrition and effective management of diseases, lab-reared colonies are typically more uniform and higher quality, disease-free organisms with a known rearing history (Roe *et al.,* 2017).

The first known plant-feeding insect to be reared from egg to adult on artificial diet was the European corn borer, *Ostrinia nubilalis* Hubner (Beck *et al.*, 1949). This diet subsequently formed the basis of many phytophagous insect diets (Davis, 2007). Hervet *et al.* (2016) further described the versatility of the McMorran diet, which was primarily used to rear species of Noctuidae. Adkisson *et al.* (1960) were the first to use wheat germ as an ingredient in an artificial diet to rear *P. gossypiella*. The recipe was later modified by Vanderzant *et al.* (1962) to rear the corn earworm, *Helicoverpa zea*. In 1963, Berger further modified this diet to rear several noctuid insect species. In 1965, McMorran modified this latter recipe to rear species of the tortricid family and in 1973, Grisdale consequently added linseed oil to the recipe as an ingredient to reduce wing deformities in some lepidopteran species. Based on this recipe, Atkinson (1978) successfully developed the first artificial diet for mass-production of *E. saccharina* in South Africa.

Diet development for mass-rearing is a continuous process in integrated pest management (IPM) research. An example is the development of diets for *E. saccharina* at the South African Sugarcane Research Institute (SASRI). Following the work of Atkinson (1978), Graham and Conlong (1988), Rutherford and Van Staden (1991), Gillespie (1993), Walton and Conlong (2016) and Ngomane *et al.* (2017) all produced "fit for purpose" new diets. These diets continuously improved quality and production of insects needed for various IPM programs, whilst reducing production costs (e.g. Ngomane *et al.*, 2017).

Mass-rearing of insects has expanded dramatically in the agricultural industry for development and support of IPM (Leppla *et al*. 2009). This had led to books being written outlining specific diets for different insect species (Singh 1977, Singh and Moore, 1995), on how to develop insect diets (Cohen 2015, 2020) and outlining the principles and procedures for rearing high-quality insects (Schneider 2009). These books also deal with good insectary management, mechanisation of rearing steps, pathogen contamination and control, and diet nutrient quality.

1.2. Working larval diets for rearing Lepidoptera pests

In order to develop artificial diets that are effective, knowledge of the functional aspects of the diet nutrient components needed by the insects is essential. In addition, the balance of nutrients such as carbohydrates, proteins, lipids, vitamins, and minerals in artificial diets developed for insects is important because it directly influences insect growth, tissue maintenance, reproduction, and energy allocation (Genc, 2006). Water is also a fundamental component. Water content and water activity (a_w) are essential because they help clarify how artificial diets work, why they sometimes fail, and are responsible for contamination regulation (Cohen, 2015). Other commonly added ingredients important in determining diet quality include emulsifiers and gelling agents, stabilisers, pH modifiers, preservatives, and antimicrobial

agents (Schneider, 2009; Cohen, 2015) as they influence diet toughness and texture, which may compromise the diet's nutritional value, palatability and thus consumption of the diet by the insect (Karowe and Martin, 1993; Schneider, 2009). Diet development should also anticipate processing and handling requirements within the mass-rearing system, so that the diet, its method of manufacture, and the rearing system work well together to efficiently deliver a high quality, nutritious, palatable foundation for the insect. Effective insect diets need the following:

1.2.1 Carbohydrates

Carbohydrates serve as building materials, energy sources and often act as feeding stimulants in insect diets. They are essential for optimum growth and development, reproductive activity, and survival of the insects. Also, the primary structure of an insect's body consists mainly of a polysaccharide (chitin) made of amino sugars (Genc, 2006; Schneider, 2009; Cohen, 2015). Certain phytophagous insects fail to thrive on diets that have less than 50% carbohydrates. For most insect species, glucose, fructose, and sucrose are nutritionally adequate carbohydrate sources (Rockstein, 1978; Cohen, 2015). Some carbohydrates such as cellulose cannot be digested by insects but may be useful as a bulking ingredient and help promote intestinal mobility (Cohen, 2015).

1.2.2 Proteins and amino acids

Insects require optimum levels of proteins for best growth. Most insects digest proteins (polypeptides) from their food, which get broken down into amino acid components and absorbed and distributed to cells where they are resynthesized into proteins that also make up the insect's body (Cohen, 2015). Insects use proteins as their principal source of nitrogen for structural purposes, and as enzymes for transport and storage (Genc, 2006; Cohen, 2015). Most adult female insects need protein to mature their ovaries and eggs, and males of many insect species require proteins for adequate longevity (Offor, 2010). As a rule, insects require a dietary source of 8 to 10 essential amino acids (methionine, threonine, tryptophan, valine, isoleucine, leucine, phenylalanine, lysine, arginine and histidine) and in the absence of one of these, growth and development may be inhibited (Genc, 2006; Cohen, 2015). Some amino acids are important in morphogenesis, others are known to be neurotransmitters. Proline for example is essential for development and serves as an energy source to support flight (Genc, 2006).

1.2.3 Lipids

Lipids in biological organisms consists of fatty acids, alcohols, sterols and phospholipids (Genc, 2006). These are good sources of energy, in addition to functioning as building blocks of cell membranes, hormones, nutrient transporters and structural materials (Cohen, 2015). Studies have shown that more than 50 insect species, including Lepidoptera, require a dietary source of polyunsaturated fatty acids (e.g. palmitic, oleic, linoleic oils etc.) and deficiencies in these result in wing deformities as their scales adhere to pupal cases on emergence (Genc, 2006; Schneider, 2009). Furthermore, phospholipids have been proven to increase fecundity when incorporated into artificial diets developed for phytophagous insects, and carotenoids have an effect on the coloration of certain insects (Offor, 2010; Cohen, 2015).

1.2.4 Vitamins and minerals

Vitamins and minerals play an important role in insect diets and although our understanding of their requirements is limited, insects need trace amounts of these nutrients for their functioning (Cohen, 2015). Insects cannot synthesize vitamins but require a good source of thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, folic acid and biotin (obtained directly from their host plants) as cofactors to help enzymes catalyse metabolic pathways. Nutritional deficiencies of these vitamins commonly result in poor growth rates, lowered fecundity or fertility and reduced body weight (Genc, 2006; Cohen, 2015). Minerals in insect diets are

intentionally added as salt mixtures (e.g. Beck's salt) (Cohen, 2015). Sodium, potassium, calcium, magnesium, chloride and phosphate are essential minerals for insects and function as co-enzymes in purine metabolism (Genc, 2006). The balance of these minerals was found to support the development of most corn borers and other lepidopteran insects (Offor, 2010).

1.2.5 Emulsifiers and gelling agents

Emulsifiers and gelling agents act as stabilisers, allowing lipid-phase materials and aqueous-phase materials to mix. They help preserve the mixed state of the ingredients and prevent reactions taking place between ingredients. This helps accommodate solid substrate-feeding insects and prevents food from collapsing on insects as they feed inside the diet (Cohen, 2015). There are two classes of emulsifiers in insect diets: natural ones which include proteins (e.g. egg yolk, milk and soy proteins) and phospholipids (e.g. soy lecithin) and artificial ones which include polyoxyethylenesorbitans (Cohen, 2015). Some gelling agents such as proteins, starches and pectin can be used as nutrients, while others such as agar and carrageenan gel are non-digestible but contribute as texturizing agents (Cohen, 2015).

1.2.6 Antimicrobial agents

The success of mass-rearing insects on artificial diet is limited by microbial (i.e. bacterial, yeast, mould, fungal, viral etc.) contamination. Microbial contamination alters the nutritional value of a diet, resulting in multiple effects on insect quality. These include reduction in insect health and size, prolonged development, increased mortality and reduced production of essential fatty and amino acids (Sikorowski and Lawrence, 1994; Sridhar and Sharma, 2013; Nair *et al.,* 2019). Most often protective ingredients are added to insect diets to prevent microbial contamination, oxidation or other means of nutrient destruction. These include antibacterial agents (e.g. streptomycin sulphate and chlortetracycline), antifungal agents (e.g. sorbic acid, methyl paraben, propionic acid and formalin) and antioxidants (e.g. ascorbic acid, tocopherol and butylated hydroxytoluene) (Cohen, 2015).

1.2.7 Importance of the diet's pH

The pH imparts several features to insect diets. It influences diet palatability, stability in relation to microbial contaminants, activity of preservatives, solubility of nutrients and functioning of enzymes (Karowe and Martin, 1993; Cohen, 2015). In general, insects prefer a slightly acidic pH range in the diet and they have the ability to regulate pH to support the acidic environment they encounter in the food and the intestinal pH changes caused by the type of food they ingest (Cohen, 2015; Dias *et al.,* 2019). Most antifungal agents only work at an acidic pH and bacterial growth on insect diets is also known to be suppressed at low pH (Cohen, 2015). Substances commonly used in insect diets to lower pH include hydrochloric acid, acetic acid, phosphoric acid, benzoic acid, citric acid, lactic acid, formic acid and tartaric acid. Bases used to raise diet pH include sodium hydroxide, potassium hydroxide, sodium carbonate and sodium bicarbonate (Cohen, 2015).

1.2.8 Water content (%) and water activity (a_w)

Most organisms need water, either obtained from their food or from a drinking source, to sustain life processes. In insect rearing programs, unintentional creation of water stress can be disastrous and lead to shortcomings in insect rearing (Cohen, 2015). Insect artificial diets should contain the normal amount of water present in the insect's natural diet. For example, certain species of leaf feeders (i.e. cabbage loopers or beet army worms) are adapted to food that is about 90% water and at anything less than that, insects would be stressed (Cohen, 2015). High nitrogen content in the diet also increases water stress, even if the water percentage is right. Such a diet can cause an insect to get rid of excess nitrogen waste forcing it to excrete

excessive amounts of water. Conversely, providing too much water can result in nutritional stress for insects adapted to feeding on diets that are concentrated in nutrients (Cohen, 2015).

Water activity (a_w), plays an important role in the diet's stability, shelf life, handling characteristics, physical properties, susceptibility to microbial contamination and chemical stability (Cohen, 2015). Free water in a diet supports microbial growth, participates in chemical and enzymatic reactions and supports spoilage processes (Rockland and Nishi, 1980). Water activity values range from 0.00 to 1.00 (a_w of water is 1.00) and according to Rockland and Nishi (1980), the water activity level known to limit bacterial growth is $0.90a_w$, $0.70a_w$ for spoilage moulds and the lowest limit for microorganisms such as yeast is $0.60a_w$.

1.2.9 Diet preparation

There are several flexible ways of preparing artificial diets for insect rearing, however, very few of these are common practices. Dyck (2010) provides a general guideline of methods for preparing insect artificial diets. Heating plays an important role in destroying microbial contaminants, detoxifying soy proteins, activating starch formation and gelling reactions and hydrating fillers such as soy meal (Dyck, 2010). Heating water between 52˚C up to 100˚C and adding gelling agents such as agar to the boiling water is required. Vitamins such as ascorbic acid should be added at temperatures not more than 60˚C. Carbohydrates such as sucrose, wheat germ and flour need to be added in the diets at temperatures between 52˚C and 90˚C. Some ingredients such as ethyl-hydroxyparaben and sorbic acid should be dissolved in ethyl alcohol and ascorbic acid needs to be dissolved in water (Dyck, 2010). Other diets are covered with wax film to prevent dehydration (Stenekamp, 2011).

1.2.10 Storage of diet ingredients and finished diets

Storage temperature is one of the most important factors affecting the stabilities of diet ingredients and completed diets. In general, storage at low temperatures (2-10˚C) is preferred to storage at high temperatures (Cohen, 2015). At high temperatures, changes in stored food such as syneresis (expulsion of liquid from one compartment to another), microbial degradation, oxidation, enzymatic and non-enzymatic chemical reactions and desiccation takes place, and at low temperatures microbial growth and the rate of degrading chemical reactions is reduced (Cohen, 2015). Light is also destructive to most diet ingredients and therefore, desirable storage conditions are cold, dry and dark places with low oxygen levels (Cohen, 2015). High water activity (a_w) influences the reactivity to oxygen and contributes to the destruction of nutrients in completed diets (fully hydrated diets) (Rockland and Nishi, 1980; Cohen, 2015). Thus, to further stabilise storage, it is important that water activity is lowered (Cohen, 2015). Changes in the diet's appearance, aromas, mouth feel and taste displays changes in the nutritional quality of stored food, and insects feeding on these diets have a natural sense of what is potentially harmful to them (Cohen, 2015).

For most dry ingredients ($a_w < 0.50$, moisture content < 10%), storage at < 0˚C should preserve their nutritional value and palatability for months (Cohen, 2015). However, storing complete diets or ingredients with high water content at temperatures below 0˚C, have consequences, some of which arise from the choice of storage equipment. These consequences include (1) sublimation (water evaporation from its frozen state), which could be avoided by storing frozen materials in tightly packaged, waterproof containers and also by reducing storage time of the diets and ingredients, and (2) Freezing, which degrades diets and ingredients by separating water from solutes, causing changes in pH concentrations and access of enzymes to substrates (Cohen, 2015). Freezing also forms ice crystals, which disrupt the integrity of the naturally protective compartments that characterize diet components, thus affecting the shelf life of the diet (Cohen, 2015).

1.3. Protocols for pathogen monitoring and management.

As discussed in 1.2.6, microbial contamination poses a real and constant threat to insect mass-rearing systems. In mass-rearing systems for Lepidoptera, microbial contamination, either directly or indirectly, are the most common cause of failures to meet production targets (Stewart 1984). Pathogens can be described as micro-organisms capable of producing diseases in mass-reared insect colonies under normal conditions. Pathogens can be viral, like the *Cydia pomonella* granulosis Virus (CpGV), fungal, like *Aspergillus niger*, or bacterial, like *Bacillus thuringiensis* (Dyck, 2010). Bacteria and fungi can produce toxins which harm the insects being reared, or they may change the physical and chemical/nutritional properties of the larval diet (Sikorowski 1984), while viruses directly attack the insects. Virus are particularly challenging because they can remain covert in the reared population and can be transferred vertically through multiple generations, with little or no measurable impact on insects, until the insect colony experiences a stress and the virus infection manifests itself (Cossentine *et al*. 2005).

Diets can be sterilized by cooking, autoclaving, adding anti-microbial compounds, and pH management (Dyck, 2010). However, each of these approaches has their own particular set of drawbacks, (e.g. cooking may alter nutritional properties or additives may be harmful to the insects themselves). Furthermore, sterilizing diet alone is not enough to keep a mass-rearing facility free of pathogens. Movement of workers within and between areas in a rearing facility poses a significant threat of microbial contamination. As such, steps must be taken to wash/sterilize/sanitize people and equipment, designate clean and dirty areas, and carefully manage the flow of insects, air, materials, and personnel through these areas to minimize contamination (Dyck, 2010).

As each species of Lepidoptera targeted for mass-rearing has its own compliment of microbial pathogens and will require a unique diet, rearing conditions, and rearing systems, species specific protocols for pathogen management will need to be developed. As microbial contamination can so easily lead to production shortfalls, it is essential to develop the tools needed to identify, monitor and manage pathogens at an early stage of mass-rearing development to help keep rearing programs on schedule and within budgets.

2. Scale and Adapt Basic Rearing Technologies to Mass-rearing Systems for the Application of SIT Against Lepidopteran Pests

2.1. Mass-rearing systems for pilot-field and/or commercial scale production.

The implementation of SIT in area wide programmes bases its success on sufficient numbers of sterile moths that can compete effectively with wild moths. However, prior to effective implementation, certain validation stages are required that are also based on the stable and permanent release of sterile moths that have adequate quality and competitiveness characteristics throughout the season.

The validation step defines the main action parameters (release rates, capture and recapture ratios (moths per trap per day), release frequencies, release systems, etc.), as well as a suitable monitoring system that allows a correct evaluation of the abundance of the pest over time. Sampling systems give information about the control level under a scenario of SIT and integration with other control systems such as mating disruption (MD) and biological control (BC).

Therefore, the mass-rearing system is the heart of SIT's success in the implementation or validation stages.

Mass-rearing follows a series of sequential processes, which need to be completed in the shortest possible time to optimize the production chain. Mass-rearing processes involve the

preparation of specific artificial larval diets, the collection of eggs from an established laboratory colony, larval development, and pupal development. Finally, after adult collection it is important to consider moth immobilization through cooling, efficient and effective packaging, and irradiation before delivery. At the same time, all the processes are developed inside specific facilities with specific construction system, specific construction materials, and a clear separation of clean and dirty areas. The facility offers specific environmental conditions that must satisfy the requirements of the mass-rearing processes. Monitoring and strict control of materials, the levels of environmental contamination, and the components of larval diet are critical in the case of Lepidoptera rearing. As in all rearing systems, human resources are permanently involved in the handling of biological material, production materials, and supplies. Then a specific protocol related to human flow inside the facility will minimize the potential for contamination.

Larval diet is a critical resource that must be adjusted based on the quality of its components, as well as its stability over time. The complexity of the composition of larval diet requires special attention, with specific protocols related of ingredients handling, diet preparation and delivery on the larval rearing units. Protocols must be prepared and followed. Also considering the extension of larval development, the stability of the diet is critical, so the automation systems of the diet preparation processes will facilitate healthy larval feeding, leading to high recovery rates that will impact on the cost of production.

Collection of the eggs in or on a suitable medium, their handling and sanitation protocol are essential to facilitate the health of the diet, allowing the neonatal larvae to have the essential nutrients from their hatching and their development.

Maintaining optimal environmental conditions in terms of temperature, humidity, quality, and air renewal conditions facilitates the safety of the diet, prevents the introduction of pathogens, and assists with maintaining diet consistency. Smart environmental control systems and adequate maintenance will allow minimum interference from the personnel in the rearing rooms and will reduce environmental changes, avoiding contamination.

Ideally, mass-rearing systems should remain in full production for as long as possible, to amortize the fixed costs related to the facilities and equipment, and also optimize the use of human resources.

For this, backup systems must be available for air conditioning equipment and the specific rearing equipment to give continuity to the mass-rearing process.

On the other hand, equipment maintenance and an adequate rotation of the mass-rearing staff is essential in ensuring constant production and reducing health and safety risks associated with insect rearing. Moth scales and other insect debris that can be powerful allergens and can be corrosive to the equipment. They must be controlled to protect the workers and keep equipment running.

Mass-rearing systems are subject to a certain loss of competitiveness over time. Proper colony renewal can ensure competitive offspring in the field.

In the case of Lepidoptera rearing, the possibility of counter-seasonal provision to users in the other hemisphere can result in a significant return on investment.

2.2. Methods for marking mass-reared sterile Lepidoptera.

Marking mass-reared insects is a critical element of the practical application of the SIT. Differentiating sterile and wild insects is critical for wild population monitoring, sterile:wild ratio monitoring or management, conducting quarantine activities, and declaring successful eradication (if that is the ultimate programme goal). Insect marking techniques are used across

many fields of entomology and have been reviewed comprehensively (Hagler and Jackson 2001, Hagler and Miller 2002). Marking techniques for mass-reared sterile Lepidoptera fall into two main categories: internal marking and external marking. Each of these techniques have strengths and weaknesses.

2.2.1. Internal marking techniques

Internal marking techniques are used in many AW-SIT applications against lepidopteran pests (e.g. codling moth, pink bollworm, etc.). Calco red, a plant-based dye that is commonly used, is a fat soluble dye that is mixed with the larval diet and incorporates into the insect's fat body as the diet is consumed. Procedures for incorporating this dye into a codling moth larval diet are outlined by Dyck (2010). For the pink bollworm the addition of 0.010-0.015% wt/v of calco red to the diet had no effect on development, adult longevity, fecundity, or mating. The appropriate dye and concentrations of dye in the diet must be determined for each species. Too little dye in the diet and the insects will not be marked strongly enough to be identified in the field. Too much dye in the diet and this additive may have adverse effects on the insect's biology or behaviour. Calco red has the added disadvantage that insects must be crushed to be identified which destroys the specimen and can be time consuming.

2.2.2. External marking systems

External marking systems are also commonly used to identify adult Lepidoptera from mass-rearing facilities. These marking systems could be the main technique for differentiating sterile insects from wild insects or they can be used to evaluate modifications to the rearing process under open field conditions (i.e. mark/release/recapture experiments). External markings most commonly come in the form of fluorescent powders (zinc-8 hyrdoxyquinoline) and are available in several colours of dyes. Insects marked with these powders are then observed under longwave UV light (Dyck 2010). Machinery and protocols for applying these powders have been developed (Logan and Proverbs 1975). Small doses of fluorescent powders (0.2 mg powder / 1 gram of insect) have been successfully used in many field trials evaluating different rearing strategies and radiation doses in field mark/release/recapture studies (Bloem *et al*. 2007). However, the addition of too much powder can have a negative impact on insect sensilla and reduce competitive ability (Stephens *et al*. 2008) or certain types of powders may have adverse impacts on insect biology.

The protocols and equipment will need to be evaluated for each new species of Lepidoptera undergoing marking, as different morphology and physiology must be taken into consideration. As AW-SIT programmes are developed for different lepidopteran pests, it is likely that both of these techniques will need to be adapted to conduct the programme and associated research.

2.3. Handling protocols and packing materials for mass-reared Lepidoptera.

The ability to produce and package sufficient high-quality insects is essential to the success of a sterile insect release programme. Effective handling protocols and appropriate packaging material are key components to the overall quality of the end product and its cost. In several lepidopteran SIT programmes, the adult moths are chilled immediately after collection (Bloem *et al*., 2007; Hofmeyr and Pretorius, 2010). This is done to immobilize the moths for packaging, irradiation, and transport which both improves the ease of handling and helps to reduce any quality degradation. The way in which the moths are handled and packaged can have a marked impact on the overall quality and fitness of the moths (Calkins and Parker, 2005).

2.4. Technology for mechanically separating mass-reared male and female moths, particularly for those species with female choice mating systems.

The key component of a conventional SIT program is sterile or semi-sterile males for release. Thus, sex separation to obtain males only for irradiation and release could improve overall efficiency (Papathanos *et al*., 2009). Development of a sex separation technique to obtain males only during or after production, will lead to lower production and post-collection handling costs, as less material and space will be taken up for a given number of sterile males (Papathanos *et al*., 2009). Females obtained during adult sex separation can also be added back into the brood-stock colony. Furthermore, there is the possibility of preferential mating between released steriles and the fact that sterile females do not directly diminish wild populations by inducing sterility (Vreysen *et al*., 2006). A possible benefit of released sterile females may be that they can distract wild males and act as a "sperm sink" (Hight *et al*., 2005).

Sex separation techniques can be grouped into two main categories viz. biological and genetic methods (Papathanos *et al.,* 2009). Genetic methods include the development of genetic sexing strains, sex-specific repressible lethality, fluorescent markers, and transgenic methods. Biological methods include sexual dimorphism at various life stages such as weight or volume differences (Serfontein, 2020); visual differences such as pupal shape and 5^{th} larval instar coloration (*Lobesia botrana*) (Steinitz *et al.* 2016); pupal colour (*Ceratitis capitata* Wiedemann (Tephritidae; Diptera)); protandry (difference in developmental time); and behavioural differences at various life stages, such as male swarming.

2.5. Optimize egg collection, processing, sanitation, incubation, and diet seeding methods and technology.

Egg production and collection is a foundational component of a mass-rearing system. Insect eggs and their handling and seeding methods impact both the potential size of the mass-rearing operation and also the health and quality of the insects produced. Egg production enclosures should be designed to take advantage of the natural behaviors of the insect species when possible, and must facilitate collection of eggs efficiently and in a way that anticipates the subsequent handling, disinfection, and production seeding processes. An example of challenges to utilizing natural insect behavior is navel orangeworm's propensity to lay eggs along a line or seam such as is found on the splitting hulls of pistachio and almond nuts. This behavior makes square or box shaped containers challenging, as the insects may lay their eggs along the joints of two sides of the box rather than on the desired substrate. Such behaviors may be able to be used to advantage with the right container design.

Egg collection substrates should also anticipate handling downstream in the rearing process. Disinfection of eggs has proven critical to many mass-rearing systems, as any pathogens which are carried on the surface of the eggs will be brought into the entire production system. Surface disinfectants must be explored that will provide the necessary pathogen control without adversely impacting neonate health. Formalin has proven an effective disinfectant in many applications, but regulatory and other constraints prevent its use in some countries. Hydrogen peroxide-based surface disinfectants, as well as chlorhexidine gluconate, have been used or tested in some mass-rearing systems, and may be viable alternatives.

As part of environmental controls overall, egg incubation is an important part of an effective mass-production system. Commercial-scale production is predicated on timing, and the reliability of production dates and times throughout the process. Egg incubation in a controlled environment helps provide consistent and reliable egg development and enhances the precision timing of processes further along in the rearing system.

Seeding diet or production containers with the desired number of eggs is critical to production output and moth health and quality. Furthermore, it offers an area of potential process automation. Eggs may be manually implanted into production containers on the egg-laying substrate (egg pads) collected from egg production cages, which requires quantification of egg count on each pad and perhaps manual cutting in order to obtain approximately the right number of eggs. Over- or under-seeding poses challenges ranging from low production output and inefficiency to disease outbreaks or potential insect cannibalism. Quantification of eggs and cutting or otherwise distributing them in diet for production offers a potentially large efficiency improvement if it can be successfully developed and implemented.

2.6. Document forecast(s) and economic analysis of mass-rearing and handling systems for the application of AW-SIT.

Economic analysis of mass-rearing cost is derived from a dynamic economic matrix that must accurately quantify each stage of the process. Starting from a clear definition of the process, the cost of each "module" of the rearing process can be generated. Thus, costs associated with each module for human resources, critical components (larval diets components), equipment involved, and the impact that each of them has on the rearing process can be defined. Finally, the contribution of each module to overall rearing costs can be determined.

A study of constraints on the mass-rearing system must be conducted, and once constraint points that affect cost are identified, they are verified, analysed, and improved. After that, other components are taken into consideration.

The dynamics of production cost is intimately related with the possibilities of use of the sterile insects in the field. Managing the seasonality of production is critical in improving the cost per million of insects. The commercialization of satellite production systems that allow users to have basic biological material available for their multiplication could increase the possibilities of production in contrast season. The production of insects from the provision of eggs in other species such as fruit flies is well known. However logistic studies should be carried out.

The permanent monitoring of different parameters of the economics of users is not a minor issue in countries with fluctuating economic systems, especially due to the weakness of their currencies and the depreciations it suffers. An economic equation must be established that allows permanent monitoring of such variables as well as the competitive possibilities of SIT with other control tools or detect SIT opportunities.

3. Optimize Existing Mass-rearing Systems for Lepidoptera to Increase Efficacy and Reduce Cost of Existing SIT Programmes.

3.1 Refined and optimized larval diets for the more efficient and effective application of existing AW-SIT programs.

Larval diets are a critical component of Lepidoptera mass-rearing systems. As described in sections 1.1, 1.2, and 1.3 there are many elements that can influence the quality of an insect's diet. While a working diet may be developed and adopted for an AW-SIT programme, improvements may still need to be made. For example, the AW-SIT programme against the codling moth in Canada tripled the moth output of their rearing facility from 5 million to 15 million insects per week, without increasing the size of the rearing facility (Dyck, 2010). This remarkable achievement was accomplished by optimizing larval utilization of the diet, improving environmental conditions within the diet, and by reducing/eliminating microbial contamination (Dyck, 2010).

Improving production capacity is not the only reason to modify and refine a working larval diet. Formalin (formaldehyde) is a potent anti-microbial agent used in many lepidopteran larval

diets (Dyck 2010). However, formalin is also a potential human carcinogen. Alternatives to formalin are desirable to increase worker safety in rearing facilities.

Improvements in rearing processes and automation may require minor or significant changes to larval diets. For example, automated diet preparation and extruding machines can significantly increase larval diet consistency, sterility, and reduce production costs. However, changes to the larval diets will likely be needed to ensure insect quality. Automation is further discussed in section 3.2.

Improving the field competitiveness of sterile Lepidoptera may also be an objective of refining larval diets. As our understanding of insect physiology, nutrition, and mass-rearing increases, additional ingredients that enhance diets will be discovered. Procedures and technology to incorporate diet enhancing additives are discussed in section 3.3 below.

3.2. Increased automation of mass-rearing processes.

Automation in mass-rearing processes offers great potential for improved use of labor and particularly for consistency of operation. Automation in diet production, such as through the use of twin-screw or similar extrusion technology, can provide a significant advance in diet consistency and a dramatic reduction of time required to produce diet. In the pink bollworm program in the USA, diet production time was reduced from days to minutes, batch consistency was significantly improved, and space requirements were significantly reduced. It also allowed the program to expand further with the limited available labor force.

Automation in egg handling and seeding offers the potential for improved process consistency and accuracy as well. For example, if the quantification, preparation, or seeding of eggs into diet could be automated, a great deal of process improvement throughout the production system may be gained via improved consistency. Automation in moth collection may offer the ability to collect insects at times of the night when labor is difficult to obtain, or it may allow collection systems to be triggered by fill level or other means. Similar benefits may be available from automating the filling of transport and release containers. Moths are highly susceptible to damage from rough or improper handling, and automation offers the possibility to reduce this damage and thus improve the health and quality of the moths.

Automation also offers potential benefits to the overall hygiene and sanitation programme in a mass-rearing system. Cleaning and disinfection of production equipment and containers may be able to be improved through the consistency and lack of handling (such as with potentially contaminated gloves) offered by automated cleaning systems. Design and construction may be challenging but may be justified by reduced pathogen pressure.

3.3 Procedures and technology to incorporated diet enhancing additives.

To develop effective artificial diets, a knowledge of the functional aspects of the diet nutrient components needed by the insects is essential. In addition, the balance of nutrients, i.e. carbohydrates, proteins, lipids, vitamins & minerals, in artificial diets developed for insects is important because it directly influences insect growth, tissue maintenance, reproduction and energy allocation (Genc, 2006). The concepts of water content and water activity are responsible for contamination regulation (Cohen, 2015). Gelling agents, stabilisers, pH modifiers, preservatives, and antimicrobial agents influence diet toughness and texture. This may compromise the diet's nutritional value and palatability affecting the insect's consumption of the diet (Karowe and Martin, 1993; Schneider, 2009; Cohen, 2015).

The diet expense is generally higher than insect production costs, therefore it is essential to avoid over-supply of nutrients (De Goey, 1973; Sahtout, 2012), which can also contribute to the build-up of primary or secondary metabolites. Furthermore, undersupply or the absence of

nutrients for the reared insects may lead to a total breakdown in production. To minimize these shortfalls in diet production, techniques have been developed to evaluate actual nutritional requirements of the animal or insect to be fed, to help better formulate insect diets (Woods *et al*., 2019b).

The carcass milling technique plays an important role in the development of animal feeds and more recently, insect diets. This technique requires that representative insects (and their natural food) are slaughtered and analysed for dry matter, crude protein, crude fat, and energy using proximate and amino acid analyses (Babinszky and Barsony, 2013; Woods *et al*., 2019b). Information collected from the proximate and amino acid analyses is then used in a feed formulation programme (WinFeed) to develop relatively inexpensive artificial diets (Woods *et al*., 2019b).

Continuous rearing on multiple generations on an artificial diet can influence insect survival rate, growth rate, fecundity, fertility and hatching success. Difficulties in molting under certain environmental conditions have also been noted by Babu *et al*. (2018). The following are possible factors that could mitigate this. Sterols greatly influence insect growth and development. The most dominant sterol in animals is cholesterol. In plants, small amounts of cholesterol are present but their dominant sterols are phytosterols (e.g. sitosterol, stigmasterol) (Janson *et al*., 2009; Bouvaine *et al*., 2014). Unlike most animals, insects are unable to synthesize sterols de novo, and depend directly on a dietary supply of these nutrients (Jing *et al*., 2013; Babu *et al*., 2018). Cholesterol incorporated into the diet usually satisfies the sterol requirements for most insect species, even in trace amounts (Behmer and Nes, 2003). Studies have shown that diets containing phytosterols, sitosterol, and stigmasterol positively influence lepidopteran species' rearing development.

A high quality, reproductive insect reared in a mass-rearing facility could easily be a poor performer in the field, especially since reproductive output and field performance such as survival under stressful environmental conditions, flight ability and mating competitiveness might be traded off. So, for most control methods in IPM research, some form of quality control or 'filtering' for specific trait(s) is required to aid and/or enhance effective field performance or other required fitness traits of laboratory reared insects (Chidawanyika and Terblanche, 2011; Sorensen *et al*., 2012).

One crucial characteristic of climate identified to potentially limit species distribution is low-temperature performance or tolerance (Kleynhans *et al*., 2014). In tropical areas, insects exposed to 10-15 °C may result in a chill-coma (cold-induced paralysis) or death, whereas insects in temperate and polar-regions may remain active and are able to fly at much lower, even sub-zero temperatures. According to Kostal *et al*. (2011), the process of cold acclimation and freezing tolerance of insects generally involves significant biochemical changes, such as rapid increases in concentrations of cryoprotectants (e.g. proline and trehalose) and increases in relative proportions of phospholipids that couple palmitic and linoleic fatty acids in cell membranes. The rapid increases in these likely contribute to the preservation of proteins and membrane structures and insect function at low temperatures (Kostal *et al*., 2011).

INDIVIDUAL PLANS

To achieve the long-term goals posed above, participants in this RCP have committed to pursue research on three sub-themes. The three research themes will be addressed by using similar approaches on different Lepidopteran species.

List of Research Themes Carried by Agreement/Contract Holders

Research Themes		**Nilo Oueyt**	**Susana Izquierdo**	**George Saour**	**Charles Burks**	**Eoin Davis**	**Evan Esch**
Basic rearing	Basic rearing technology		*L. botrana*				
	Working larval diet(s)						
	Pathogen Management			*L. botrana*	*A. transitella*	***A. transitella***	
Scaling	Mass-rearing systems	***L. botrana***					***C. pomonella***
	Marking Methods	*L. botrana*					
	Handling and Packing	*L. botrana*	***L. botrana***		***A. transitella***	*A. transitella*	
	Male and Female Separation				*A. transitella*		
	Egg Collection and Diet seeding	*L. botrana*	*L. botrana*			*A. transitella*	
	Document Forecast & Economic Analysis	*L. botrana*					*C. pomonella*
Optimizing	Refined & Optimized Larval Diets	*L. botrana*	*L. botrana*	*L. botrana*		*A. transitella*	
	Increased Automation	*L. botrana*	*L. botrana*			*A. transitella*	*C. pomonella*
	Diet Enhancing Additives			***L. botrana***		*A. transitella*	

List of Research Themes Carried by Agreement/Contract Holders (Count.)

Research Themes		**Megan Mulcahy**	**Lawrence Malinga**	**Shiva Osouli**	**Muhammad Sarwar**	**Hancheng Wang**	**Nalini Behary Paray**
Basic rearing	Basic rearing technology				*H. armigera*, *S. litura*	*S. litura*	*C. sacchariphagus*
	Working larval diet(s)				*H. armigera*, *S. litura*	*S. litura*	*C. sacchariphagus*
	Pathogen Management				*H. armigera*, *S. litura*	*S. litura*	*C. sacchariphagus*
Scaling	Mass-rearing systems		*E. saccharina*				*C. sacchariphagus*
	Marking Methods			*H. armigera*			*C. sacchariphagus*
	Handling and Packing	*T. leucotreta*	*E. saccharina*	*H. armigera*			*C. sacchariphagus*
	Male and Female Separation		*E. saccharina*				
	Egg Collection and Diet seeding		*E. saccharina*	*H. armigera*			
	Document Forecast & Economic Analysis	*T. leucotreta*					
Optimizing	Refined & Optimized Larval Diets						
	Increased Automation	***T. leucotreta***	*E. saccharina*	*H. armigera*			
	Diet Enhancing Additives	*T. leucotreta*		*H. armigera*			

List of Research Themes Carried by Agreement/Contract Holders (Count.)

Research Themes		**Jerome Niogret**	**Savantil Anisah**	**Stefan Foord**	**Hien Thi Thanh Nguyen**	**Maohua Chen**	**Xiaoxia Liu**
Basic rearing	Basic rearing technology	*C. cramerella*	*C. cramerella*	*T. batrachopa*	*O. arenosella*	*C. sasakii*	*G. molesta*
	Working larval diet(s)	*C. cramerella*	*C. cramerella*	*T. batrachopa*	*O. arenosella*	*C. sasakii*	*G. molesta*
	Pathogen Management	*C. cramerella*	*C. cramerella*				
Scaling	Mass-rearing systems				*O. arenosella*		
	Marking Methods						
	Handling and Packing			*T. batrachopa*			
	Male and Female Separation						
	Egg Collection and Diet seeding						
	Document Forecast & Economic Analysis						
Optimizing	Refined & Optimized Larval Diets						
	Increased Automation						
	Diet Enhancing Additives						

LOGICAL FRAMEWORK

Project Design Elements	**Objective Verifiable Indicators**	**Means of Verification**	**Important Assumptions**
Overall Objective Expand and improve the sterile insect technique for use against lepidopteran pests of crops to improve food security and enhance health and well-being.	N/A	N/A	The absence of basic and mass rearing methodology is a roadblock to the application of SIT against many pests. With basic and mass scale rearing technology in place, expertise, resources and financial support to pursue AW SIT will be available in member states. Increasing the efficiency of efficacy of existing rearing systems will ensure the long-term success of SIT prorgrammes.
Specific Objectives 1. Develop basic rearing technology and systems for lepidopteran pests of concern.	Working diets and rearing systems for Lepidoptera demonstrated.	Reports, protocols and published papers.	Managerial support and availability of expertise and resources required conduct basic research on Lepidoptera pests.

Project Design Elements	Objective Verifiable Indicators	Means of Verification	Important Assumptions
2. Scale and adapt basic rearing technologies to mass-rearing systems for the application of SIT against lepidopteran pests.	Implement mass rearing systems for lepidopteran pests. Demonstrate meaningful increases in facility production and/or conduct pilot-field studies.	Reports, protocols and published papers.	Managerial support and availability of expertise and resources required to advance incipient area wide mass rearing programs for the application of the SIT. Pilot project approval will occur.
3. Optimize existing mass-rearing systems for Lepidoptera to increase efficacy and reduce cost of existing SIT programmes.	Increased colony insect competitiveness, reduced rearing costs, and increased programme efficacy.	Reports, protocols and published papers.	Managerial support and availability of expertise and resources required to research, implement, and evaluate optimization of mass reared Lepidoptera
Outcomes			
1. Mass-rearing technologies as a foundation for AW-SIT and other pest management strategies developed.	Advances toward the application of SIT for lepidopteran pests.	Technical reports and papers published documenting diet development.	Improved technology and methods adopted by member states.
2. Mass-rearing systems to integrate SIT into AW-IPM programmes expanded.	Increased production, systems procedures, technology, protocols	Technical reports and papers published documenting diet and rearing systems.	Improved technology and methods adopted by member states. AW SIT will be cost effective.

Project Design Elements	Objective Verifiable Indicators	Means of Verification	Important Assumptions
3. Efficacy and efficiency of established AW-IPM programmes using SIT against lepidopteran pests increased	Improved indices of sterile moth performance, S:W ratios, reduced production costs, reduced crop loss, and overall increased program efficacy.	Technical reports and papers published documenting increased efficiency and efficacy of rearing technology. Increased production, reduced costs, increased trap captures.	Improved technology and methods adopted by member states.
Outputs			
1.1. Develop basic rearing technology for candidate pests.	Protocols, technology, and systems for rearing Lepidoptera pests	Reports and or published protocols	This technology will be scalable to mass production systems and will lead to further development of SIT for that pest.
1.2. Working larval diets for rearing Lepidoptera pests.	At least one diet demonstrated to be suitable for rearing.	Reports and or published protocols	Diets will be cost effective and scalable to mass rearing.
1.3. Protocols for pathogen monitoring and management.	Identify key pathogens and identify protocols for pathogen monitoring and management	Reports and or published protocols	Resources for pathogen identification available.
2.1. Mass-rearing systems for pilot-field and/or commercial scale production.	Demonstration of mass technologies for rearing systems for at least two elements of the rearing infrastructure.	Reports, published papers, design schematics	Resources and support for continued development of mass rearing technology. Industry will continue pilot-project support.

Project Design Elements	Objective Verifiable Indicators	Means of Verification	Important Assumptions
2.2. Methods for marking mass-reared sterile Lepidoptera.	Marked insects reliably distinguished from wild population.	Reports, published papers, or protocols.	Existing marking technology can be easily transferred to other lepidopteran species. Reliable trapping technology is available.
2.3. Handling protocols and packing materials for mass-reared Lepidoptera.	Protocol and materials demonstrated.	Reports, published papers, or protocols.	Resources and support for continued development of packaging and handling protocols.
2.4. Technology for mechanically separating mass-reared male and female moths, particularly for those species with female choice mating systems.	Reliable separation of > 95% of mass reared insects.	Reports, published papers or schematics.	Male only release continues to be a viable strategy for lepidopteran species with female choice mating systems.
2.5. Optimize egg collection, processing, sanitation, incubation and diet seeding methods and technology.	Increased efficiency, reduced costs, increased production, reduced pathogen load.	Equipment, technology, schematics, reports, published papers.	Methods and resources for QC assessments are available.
2.6. Document forecast(s) and economic analysis of mass-rearing and handling systems for the application of AW-SIT.	Cost analysis produced.	Report and/or financial audit	Reliable data is available for economic analysis and cost projections.

Project Design Elements	Objective Verifiable Indicators	Means of Verification	Important Assumptions
3.1. Refined and optimized larval diets for the more efficient and effective application of existing AW-SIT programs.	Increased production, recapture, increased QC indicators.	Reports, program measures.	Apparent improvements in moth quality can be from isolated from environmental conditions and grower practices.
3.2. Increased automation of mass-rearing processes.	Increased insect production, improved QC indicators, reduced costs.	Reports, Schematics, manuals	Automation technology will be more reliable and cost effective than unskilled labor.
3.3. Procedures and technology to incorporate diet enhancing additives.	Increased recapture under adverse field conditions.	Reports and published papers.	Diet additives will have no negative effects
Activities			
1. Announce project amongst established entomologists working in Lepidoptera AW-SIT operational programmes	Proposals evaluated and 10 Research Contracts, 10 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 (Q3 2022, Vienna, Austria.)	1st RCM held Q3 2022.	Participants' activities and logical framework revised.	Contracts and Agreements signed by counterpart organizations.

Project Design Elements	Objective Verifiable Indicators	Means of Verification	Important Assumptions
3. Provide necessary research protocols to contract holders	Research protocols available	Reports and protocols	Research protocol will be implemented by qualified scientists. Methods and resources available.
4. Conduct applied research and development	New knowledge created on Lepidoptera rearing	Scientific papers and reports from the participants	Contracts and Agreements properly managed by counterpart organizations. Methods and resources available.
5. Organize second RCM to analyse progress in delivering research outputs and plan the next phase of the project (Q3 2023).	2nd RCM held Q1 2024. Canada Q2 2024 (TBC) South Africa Q1 2024	Participants and RCM Progress Reports.	Progress satisfactory.
6. Organizing a workshop in conjunction with Second RCM (Q4 2023) Workshop replaced by diet and host chemical analysis	Workshop on Carcass milling technique held in conjunction with 2nd RCM.	Number of participants in the workshop	Funding and venue available
7. Conduct applied research and develop ment	New knowledge created on Lepidoptera rearing	Scientific papers and reports from the participants Report	Contracts and Agreements properly managed by counterpart organizations. Methods and resources available.
8. Review the CRP after its third year	Satisfactory progress of research agreements and technical contract	Participants and RCM Progress Reports.	Progress satisfactory and mid-CRP evaluation approved by CCRA-NA committee.

Project Design Elements	Objective Verifiable Indicators	Means of Verification	Important Assumptions
9. Organize third RCM to analyse progress in delivering the research outputs and plan the final phase of the project. (Q2 2025)	3rd RCM to be held Q3 2025.	Participants and RCM Progress Reports.	Progress satisfactory.
10. Conduct applied research and development	New knowledge created on Lepidoptera rearing	Scientific papers and reports from the participants	Methods and resources available
11. Organize final RCM to assess the success of the CRP in reaching its objectives and review the final publication. (Q1, 2027).	4th RCM to be held Q1 2027.	Participants and RCM Final Reports	Final reports are submitted to the Agency.
12. Evaluate the CRP and submit evaluation report.	Satisfactory completion of research agreements and technical contract	Report	Contracts and Agreements properly managed by counterpart organizations. Methods and resources available.
13. Publish the results of the CRP in a special issue of a peer reviewed journal.	At least 15 publications accepted	Scientific publications.	Consensus can be found on appropriate peer review journal and acceptance by journal obtained.

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ANNEX 2: AGENDA

Monday, 5 September 2022 (Room MOE3)

08:00 – 09:00 Registration at Gate 1 (by underground U1 station - Kaisermühlen VIC)

09:00 – 09:10 **Rui Cardoso Pereira** Welcome & opening remarks

09:10 – 09:20 Self-introduction

09:20 – 09:30 **Daguang Lu** Goals of the meeting and agenda, CRP perspectives

Presentations of Research Proposals (Chairperson: Jerome Niogret)

09:30 – 10:00 **Evan Esch** Codling Moth Rearing at the OKSIR Programme: Opportunities and Directions

10:00 – 10:30 **Megan Mulcahy** Assessing the Use of Cyclone Separators for the Improved Collection of Mass-reared False Codling Moth Adults Post Eclosion

10:30 – 11:00 Coffee Break

11:00 – 11:30 **Eoin Davis** Key Objectives to Maximize Efficiency of Mass-Rearing Navel Orangeworm

11:30 – 12:00 **Shiva Osouli** Improve the Mass-rearing of *Helicoverpa armigera* (Lep: Noctuidae) for SIT Programmes

12:00 – 13:00 Lunch Break

Presentations of Research Proposals (Chairperson: Eoin Davis)

13:00 – 13:30 **Nilo Oueyt** Development of Mass Rearing Process for *Lobesia botrana*

13:30 – 14:00 **Susana Izquierdo Carreño** Improvements in Rearing Yields and Systems for Collecting Emerged Adults from Post-irradiated Pupae for Rearing *Lobesia botrana* in Chile.

14:00 – 14:30 **George Saour** Incorporating Vegetable Oils, Powders and Selenium as Enhancing Additives to Improve *Lobesia botrana* (Lepidoptera: Tortricidae) Larval Diet

14:30 – 15:00 Coffee Break

15:00 – 15:30 **Lawrence Malinga** Advancing the Application of the Sterile Insect Technique (SIT) by Examining the Impact of Some Processes on the Management of Sugarcane Borer *Eldana Saccharina* in South Africa

15:30 – 16:00 **Nirupa (Nalini) Behary Paray** Mass Rearing of the Sugar Cane Borer *Chilo sacchariphagus* (Lepidoptera: Crambidae) for Quality SIT

16:00 – 17:00 Group Discussion

Tuesday, 6 September 2022

Presentations of Research Proposals (Chairperson: Evan Esch)

09:00 – 09:30 **Maohua Chen** Development and Refinement of Artificial Larval Diets for the Peach Fruit Moth *Carposina sasakii*: the Best Diet for High Moth Quality and Performance Recommended for SIT Programmes (virtual presentation)

09:30 – 10:00 **Xiaoxia Liu** Establish a Suitable Diet Formula and Environmental Conditions (Photoperiod, Humidity and Temperature) to Mass-rearing *Grapholita molesta* (Busck) to Increase the Use of Sterile Insect Technique (virtual presentation)

10:00 – 10:30 **Hancheng Wang** Optimization of Rearing Conditions of *Spodopetera litura*, the Main Pest of Tobacco in Southwest China (virtual presentation)

10:30 – 11:00 Coffee break

11:00 – 11:30 **Jerome Niogret** Optimization of Rearing Conditions for the Cocoa Pod Borer, *Conopomorpha cramerella*, the Main pest of Cacao in Southeast Asia

11:30 – 12:00 **Anisah Savantil** Rearing System Development for the Cocoa Pod Borer (Lepidoptera, Gracillaridae) in Malaysia

12:00 – 13:00 Lunch Break

Presentations of Research Proposals (Chairperson: Lawrence Malinga)

13:00 – 13:30 **Stefan Foord** Develop Mass Rearing of the Macadamia Nut Borer *Thaumatotibia batrachopa* (Meyrick) (Lepidoptera: Tortricidae) for Quality SIT

13:30 – 14:00 **Nguyen Thi Thanh Hien** Study on Artificial Larval Diet for Mass Rearing *Opisina arenosella* Walker

14:00 – 14:30 **Muhammad Sarwar** Developing of Artificial Rearing Systems for Cotton Bollworms (Lepidoptera) in the Context of Sterile Insect Technique (SIT)

14:30 – 15.00 Coffee Break

15:00 – 15:30 **Des Conlong** Carcass milling technique (TBD, virtual presentation)

15:30 – 16:00 **Charles Burks** Modifications in Rearing and Transport of Navel Orangeworm for Improved Effectiveness of Sterile Insect Technique (virtual presentation)

16:00 – 17:00 Group Discussion

Wednesday, 7 September 2022

Review of Individual Proposals (Chairperson: Daguang Lu and Group Leaders)

09:00 – 10:30 Working Groups: revising individual plans for the 5 year of the CRP and for the next 18 months

10:30 – 11:00 Coffee break

11:00 – 12:00 Working Groups: revising individual plans for the 5 year of the CRP and for the next 18 months

12:00 – 13:00 Lunch Break

13:00 – 15:00 Working Groups: revising individual plans for the 5 year of the CRP and for the next 18 months

15:00 - 15:30	**Coffee break**
15:30 – 17:00	Working Groups: revising individual plans for the 5 year of the CRP and for the next 18 months

Thursday, 8 September 2022

RCM report (Chairperson: Daguang Lu and Group Leaders)

09:00 – 10:30	Review and adjustment of the logical framework **(LFM)**
10:30 – 11:00	**Coffee break**
11:00 – 12:00	Review and adjustment of the logical framework **(LFM)**
12:00 – 13:00	**Lunch Break**
13.00 – 15:00	Review and agreement on content of RCM report
15:00 – 15:30	**Coffee break**
15:30 – 17:00	Review and agreement on content of RCM report
18:00 - 20:00	Group dinner (TBD, IAEA will cover the drinks)

Friday, 9 September 2022

RCM report (Chairperson: Daguang Lu and Group Leaders)

09:00 – 10:30	Finalizing RCM report
10:30 – 11:00	**Coffee break**
11:00 – 12:00	Finalizing RCM report
12:00 – 13:30	**Lunch Break**
13.30 – 15.00	Presentation of the final RCM report (main goals and future work)

ANNEX 3: WORKING GROUPS TO DEVELOP THE DETAILED R&D PLANS FOR THE FIRST 18 MONTHS OF THE CRP

Group One (Basic rearing)	**Group Two (Scaling and optimizing)**
Mr Eoin Davis (Group Leader)	Mr Evan Esch (Group Leader)
Mr Jerome Niogret	Mr Nilo Oueyt
Ms Savantil Binti Anisah	Ms Susana del Pilar Izquierdo Carreño
Ms Nirupa (Nalini) Behary Paray	Ms Carolina Alejandra Yáñez Briceño
Mr Muhammad Sarwar	Mr Georges Saour
Mr Stefan Foord	Ms Shiva Osouli
Ms Hien Thi Thanh Nguyen	Mr Lawrence Malinga
	Ms Megan Mulcahy

ANNEX 4: ABSTRACTS OF PRESENTATIONS

Development of Mass Rearing Process for *Lobesia botrana*

Nilo Oueyt, Érica Gómez Barrionuevo, Mario Sevilla, Gustavo Taret and Mariel Vanin.

ISCAMEN (Institute of Agricultural Health and Quality of Mendoza)

Lobesia botrana (Den & Schiff) (Lepidoptera: Tortricidae), known as "European grapevine moth", was detected for the first time in February 2010 in traps located in Maipú, Province of Mendoza. As a result of the detections SENASA "National Service of Agri-Food Health of Argentina" declared the phitosanitary emergency on March 3rd of 2010, in order to contain and control the already mentioned pest. It reached 15.000 hectares in 2010 and by 2016 it was expanded to 158.000 hectares because only some private properties applied the recommended strategies as the sexual confusion and agrochemicals. Since 2016 a workplan of wide area with the concept of Integrated Pest Management is stablished together with one public-private organization and with the participation of resources of both sectors. The workplan emphasized the use of the sexual confusion technique in combination with agrochemicals of low impact managing to reduce the plague in a notorious way. By the year 2018 the populations increased due to low financial resources. Currently, this plague is present in 135.000 hectares of vines, however have been generated approx. 23.000 hectares of low prevalence or probably free of the pest where there would be the possibility of applying the SIT as long as the necessary adjustment is achieved. Furthermore, the urban zones with abundant vine plants located in the backyards would also be zones for the SIT to be applicated considering the low efficiency of the Sexual Confusion Technique and the impossibility of applying agrochemicals. Noteworthy ISCAMEN counts with a Modular Mass Rearing Multipurpose Facility, with specific rearing and sterilization equipment. It also, has qualified personnel at different levels to execute rearing processes and vast experience in the development of rearing other insects and controllers. In addition, high-tech ground and air release packaging systems are in full development. Considering the success achieved in erradicating Medfly in the Province of Mendoza, it is considered fundamental to develop the SIT for *Lobesia botrana*. At present, it is available a colony in development at experimental level. It was established in 2017 from wild moths (larvae and pupae) and was refreshed in 2019 with eggs and pupae provided by FDF (Fundation for the Fruit Development of Chile), continuing with the development of it, implementing similar procedures as in the already mentioned organization. An increase in the production was reached until March of 2020, moment in which all the activities were affected by the lockdown imposed in Argentina as a result of the Covid-19 Pandemic. Therefore, the production of *Lobesia botrana* was negatively affected. In May-June of 2021 wild moths (pupae and larvae) were collected to increase the colony, which is in diapause. It is important to mention that it has been achieved to implement the rearing at laboratory level using a similar diet formulation as in FDF, with some adaptations to the locally available supplies. Field tests have also been carried out with the use of sterile moths and sexual confusion. The parameters of production and quality obtained at present are: Egg hatching: 94% (max.97 - min87); recovering pupae/egg: average 17% (max.47 - min 2,4); postures/female: average 16 eggs/female (max.54- min 2); pupal weight: average male 5,44 mg, female 7,25 mg; days of infestation to pupae collection: average 33% (max. 46, min 25). The development of rearing *Lobesia botrana* at massive level poses a number of challenges related to an increase of efficiency in the larval diet, in order to stabilize it. In addition, an egg collection system is required at massive level, system of automatic dosage of larval diet and a pupae/adult collection system of high efficiency are needed. The systems of packaging and liberation also require a specific development for the moth. No least is the implementation of processes of sanitation and of environmental monitoring to preserve the environmental rearing conditions in an environment with low or no contamination. These needs are raised in this project and are part of the project objectives. On the other hand, the project has also considered the determination in the field of the main indicators related to the management of the SIT and its correlation with the rates and frequencies of release.

Codling Moth Rearing at the OKSIR Programme: Opportunities and Directions.

Evan D. Esch.

Okanagan Kootenay Sterile Insect Release Board, 1450 K.L.O Rd, Kelowna, BC, Canada, V1W 3Z4

The Okanagan-Kootenay Sterile Insect Release Programme (OKSIR) in British Columbia Canada has been mass-rearing and sterilizing codling moth (*Cydia pomonella* (L.)) since 1994. This rearing system uses a modified Brinton diet, constituted mostly of low cost, local ingredients, which are prepared and dispensed into "cafeteria" style trays. Insects are reared at a constant temperature under a slowly decreasing humidity regime. Insects pupate in the dried diet. Adult male and female moths emerge into a collection room and are collected with a UV light and HVAC/cyclone system. Adult moths are chilled and diverted for egg production or irradiated for release. Codling moth rearing has been studied extensively and thoroughly reviewed. Much effort has been devoted to using diapause, fluctuating rearing temperatures and/or thermal shocks to improve competitiveness in the field, particularly under cool spring temperatures. However, because of limitations of rearing infrastructure, added costs, and lack of scientific consensus these methods have not been adopted by the OKSIR programme. Highlights of the advances to the rearing systems used by the OKSIR programme include synchronizing ambient and rearing photoperiods, improving sanitation/monitoring technologies, improving scale filtration, improving moth collection storage and handling, and automating diet preparation. Ongoing work includes automation of the collection and handling procedures and evaluation of X-ray technology for large scale rearing and irradiation. There are many potential avenues of future research including but not limited to increasing automation of the rearing process, identification of and utilization of beneficial endosymbiotic micro-organisms, evaluating x-ray technology, optimizing short, thermal shock treatments, addition of cryoprotectants to the diet, re-visiting anoxic irradiation under lower intensity X-ray irradiation. Future research on codling moth rearing should focus on reducing rearing costs and increasing competitiveness of insects, during cool, spring conditions.

Improvements in rearing yields and systems for collecting emerged adults from post-irradiated pupae for rearing *Lobesia botrana* in Chile.

Susana Izquierdo Carreño, Carolina Yañez Briceño, David Castro

Fundación Para el Desarrollo Fruticola

In 2008, the pest known as "*Lobesia botrana*" was detected in Chile in Vitis vinifera and due to the serious phytosanitary damage that this insect causes to the agricultural sector, the SAG declared it a quarantine pest under official control (Source: SAG). Its hosts include grapevines (main), cherry, olive, plum, peach, pomegranate, kiwis, berries, among others, reporting punctual and occasional attacks on some of these (Source: USDA). In the case of Chile, *Lobesia botrana*, was initially detected only in grapevine. However, in March 2014, official inspections reported detections in both blueberries and plums. Therefore, there is concern in the export sector that this pest will continue towards other fruit trees that could be in danger, such as kiwis, cherry trees, apples, which appear in the literature as secondary hosts.

This constitutes a serious risk for exports of this fruit to markets such as China, Korea, Mexico, USA, Canada, Brazil, Argentina, Peru, New Zealand and Uruguay (Source: SAG). *L. botrana*, in addition to generating direct damage due to the attack of larvae on the fruit and the subsequent appearance of rotting, has caused reductions in production, mainly related to the increase in management costs due to mandatory applications, the risk of advancing to secondary hosts and, more seriously, still, there are restrictive actions in international markets.

The demanding quarantine requirements imposed by the different international markets, such as mandatory fumigation, quarantine of regions, prohibition of the export of fruit from certain orchards

with positive detections, directly affects the condition of the fruit and its commercialization. A recent case in the 2013/14 season, of detections in blueberries, USA, determined regional quarantines and obligatory fumigation with methyl bromide, causing great losses due to the effects of fruit condition and loss of the condition of organic orchards, mainly in the VIII Region For the reasons stated above, SAG implemented a contingency plan, which has had a deployment of resources over the years, based on an integration of measures, based on surveillance, quarantine, property control and urban control.

One of the greatest difficulties since the *Lobesia botrana* plague was detected in Chile has been the elimination of foci in urban areas. In summary, *Lobesia botrana* has had negative effects on social, cost and environmental aspects, for which the need arises to investigate new biological alternatives that allow its populations to be reduced in urban areas and on organic crops.

The use of the Sterile Insect Technique (SIT) would have great potential as an additional or complementary tool in biological control programs as a sustainable and environmentally friendly technique.

This project proposes to continue the improvements in the current breeding of *Lobesia botrana*, which is kept in the FDF facilities under two fundamental precepts, to increase the efficiency of oviposition in larger areas, thus avoiding the use of such small reproduction trays, that is, say increase the production units in size, seeking to homogenize the sample. Incorporate improvements in the diet that allow lowering production costs with 5 new alternatives of artificial diets, and improve the collection of adults emerged from irradiated pupae, incorporating technology by attraction of light and action of sucked air. In such a way to avoid the harvest handling step, irradiating the pupae in the structure where they currently pupate, thus avoiding harvesting and handling them.

Optimization of rearing conditions of *Spodopetera litura,* the main pest of tobacco in southwest China

Hancheng Wang, Feng Wang, Jianyu Meng, Shenghua Shang

Guizhou Academy of Tobacco Science, Guiyang 550081, People's Republic of China

Spodoptera litura is a poly-phytophagous insect damaging several vegetables and field crops in many Asian countries including China. It could infect more than 120 host crop plants. Annual losses caused by this pest could reach $ 800 -$ 1000 M in Southwest China. So far, different artificial diets have been developed and proposed for the consistent rearing of many kinds of important pests. However, these artificial diets are still not suitable for large scale rearing of *S. litura*, and many pests frequently get diseased during feeding. The project of this study will complement the rearing environmental conditions and contamination control that influencing the rearing capability of tobacco cutworm in the next five years. The first objective of this study is to optimize the artificial diets for mass rearing of *S. litura*, and to determine the best artificial diets on the basis of its different pre-mature and adult stage biological parameters. The second objective is to identify the main constraints for mass rear of *S. litura*, and to develop some protocols to reduce the limiting factors. The third objective is to reduce the cost for mass rear of *S. litura*, and to enhance the automatic ability of mass rear of the pest. For the next 18 months, artificial diets optimizing for rearing of *S. litura* will be conducted. Meanwhile, the microbial composition of this pest will be also isolated, identified and investigated with both cultural and high-throughput sequencing techniques. Potential disease pathogens will be verified. Outcome of this study will provide large amount of tobacco cutworm which could be used for mass factory breeding of sterile insects for use in regional pest control and sustainable crop farming in Southwest China.

Development and refinement of artificial larval diets for the peach fruit moth *Carposina sasakii*: the best diet for high moth quality and performance recommended for SIT programmes

Maohua Chen, Junfeng Zuo and Sha Su

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The sterile insect technique is an environment-friendly area-wide control method which has been successfully used in suppression, eradication, containment or prevention of moth pests The peach fruit moth, *Carposina sasakii* Matsumura（Synonyms: *Carposina niponensis* Matsumura and *Carposina persicana* Matsumura) (Lepidoptera: Carposinidae) is one of the most destructive fruit borers in the orchards of deciduous fruits. This pest mainly distributes in eastern Asia countries including China, Japan and Korean Peninsula and is a quarantine pest of many countries in different continents. The larvae of *C. sasakii* tunnel all parts of the fruit, feeding on the fleshy parts and on the seeds and cause a severe economic impact every year. *C. sasakii* has been considered as a counterpart of the codling moth, *Cydia pomonella* (L.) because of their similar ecological and economic status in orchards. The latter was successfully controlled by SIT in some countries. The wide use of chemical insecticides to control *C. sasakii* has caused insecticide resistance and is increasing the residues on food and in the environment. The field populations of the peach fruit moth are predominantly relatively sedentary. The SIT can be an environment-friendly and effective control strategy for *C. sasakii*. Mass-rearing of larvae with artificial diets in the factory is crucial for the successful application of SIT against Lepidoptera. Apple fruits are used to rear *C. sasakii* in the laboratories of China. The efficient artificial diet formula for rearing larvae of this pest is still scarce, how different diets affect on the moth performance, the physiological phenotypes, and the maintenance of colonies based on selection for favourable behaviours of the pest is unknown. The objectives of this study are to develop and refine artificial larval diets for *C. sasakii*, to investigate the reproductive measures, flight capacity, cold and thermal tolerance of *C. sasakii* reared with artificial diets, to establish *C. sasakii* colonies with better reproduction measures and high male flight capacity which can be used for further SIT. The findings may have important implications for practices used for suppressing *C. sasakii* population with SIT in China and other countries.

Establish a suitable diet formula and environmental conditions (photoperiod, humidity and temperature) to mass-rearing *Grapholita molesta* (Busck) to increase the use of Sterile Insect Technique

Xiaoxia Liu, Songdou Zhang, Jie Cheng, Peng Zhao

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The oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), is a major invasive pest on Rosaceae fruit trees worldwide. Currently, the main method to control *G. molesta* is to use conventional insecticides, which can easily lead to the pesticide-resistance problem. In the past ten years, our research group has conducted much research to develop new techniques for monitoring and controlling *G. molesta* including a large number of field and indoor studies. We have monitored the dynamics of *G. molesta* in 60 orchards, distributed at 18 different provinces of China. In addition, we have explored the green control technology, such as mating disruption dispenser, sex attractants and natural enemy insects (such as *Trichogramma* and *Coccinellidae*) to control this pest. And we have developed a nanocarrier-mediated transdermal dsRNA-NPF1 delivery system that can significantly decrease the fruit damage and increase the mortality of newly hatched larvae. In order to enrich *G. molesta* effectively control methods, we plan to apply the radiation-based sterile insect technique (SIT) to control the pests in field, which has proven to be a successful strategy for pest management. However,

to successfully implement SIT on target insect pest species, methods of obtaining radiation-based sterile insects and the mass-rearing of insects need to be established first.

Therefore, in the project, our main objective is to establish the development of cost-effective and convenient artificial feeds and the exploration of suitable environmental conditions for the rapid growth and development of *G. molesta*.

Optimization of rearing conditions for the cocoa pod borer, Conopomorpha cramerella, the main pest of cacao in Southeast Asia

Arni Ekayanti[1] & Jerome Niogret[2,3]

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The cocoa pod borer (CPB) *Conopomorpha cramerella* (Snellen) (Lepidoptera: Gracillariidae) is a moth species endemic to Southeast Asia. The pest's devastating impact on cocoa farms is largely responsible for the drastic decline in the Indonesian cocoa production and other areas of Southeast Asia. It is estimated to be directly and indirectly responsible for about $300-500M in annual losses in Indonesia alone. This project will complement the knowledge of the environmental conditions and contamination control influencing the rearing capability of the cocoa pod borer.

Our first objective is the contamination control infesting artificial diet in insectaries. This includes the identification of the diet contaminant that are recurrent during the diet preparation, the discovery of antimicrobial control methodology to prevent the recurring diet contamination, and the development of egg sterilization methods to prevent contamination coming from the insect eggs onto the diet.

The second objective is to optimize the environmental conditions to improve the success rate of the artificial diet for the cocoa pod borer. Our goal will be to determine the trade-off(s) between the oviposition success rate, the hatching success rate, the larval development success rate, the pupal emergence success rate, and the adult fertility with the duration of the developmental stages. We will compare those characteristics related to various levels of humidity, lighting conditions (photoperiod), and temperature to estimate the optimal environmental conditions for CPB development.

Our third objective is to assess the competitiveness of lab-reared insects over generations (Quality Control). Evaluating the quality of our rearing conditions and the competitiveness of the insects reared on an artificial diet over several generations is the final but predominant step of this research proposal. Underdeveloped, malformed, and unhealthy insects, qualities that depend on the rearing conditions and diet composition, would have no use for further IPM research experiments.

Improve the Mass-rearing of *Helicoverpa armigera* (Lep: Noctuidae) for SIT Programmes

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H. armigera is one of the key pests of several economically important crops in Iran. The pest is widespread all over the country. Our six-year experience in laboratory rearing *H. armigera* for previous SIT research projects points to some restricted problems in the process of mass-rearing this pest for

large-scale release programs. The present research will conduct studies to enhance larval and adult insects' diets through additives aimed at improvement of reared insect quality, quantity and compatibility with release in large-scaled SIT programs. In this regard, the effect of diet additives to improve cold temperature tolerance of reared insects, developmental and reproductive parameters and sodium compensation strategies will be determined. Automation of the mass-rearing process also will be investigated by designing and constructing the egg collection cages, rearing containers, and diet dispensing facility. In addition, appropriate, easily accessible and affordable marking techniques of mass-reared *H. armigera* for released insect monitoring will be determined and an appropriate pupae packaging protocol for safe transporting to different parts of the country will be provided.

Rearing system development for the cocoa pod borer (Lepidoptera, Gracillaridae) in Malaysia

Anisah Savantil[1], Mavis Peter Jaus[1], Jerome Niogret[2,3]

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One of the major limitations of cocoa production is its susceptibility to pests and diseases. Pests and diseases related to cocoa losses occur on every continent where cocoa is grown. In Malaysia, Indonesia, Papua New Guinea and the Philippines, losses due to CPB can lead to 50-60% in yield if left uncontrolled. Various control measures have been tested in the past decades, primarily focused on breeding programs for resistance, pesticide application, sleeving the fruits, and crop management practices. None of those measures has been fully efficient due to the limited access to the pest for laboratory experiments. Rearing herbivorous insects on an artificial diet increases the insects' availability for research studies while reducing labor, time, space, and associated costs of growing the insects on their host plants. The use of artificial diet also synchronizes insect development and can be optimized and/or manipulated to increase insect fitness above their natural threshold.

Published literature on artificial diet development for CPB and on other species in its Family Gracillariidae is sparse. Perhaps the most promising diet (based on sustaining the larvae at least 11 days as the trial subsequently ceased due to contamination) developed was initiated by our group (Malaysian cocoa board - MCB) in the late 1990's. Santoso *et al.* (2004) also had similar attempts in Indonesia. Their diets were based on general Lepidoptera diets used without much success on CPB.

Today, the MCB team could get regular adult emergences based on preliminary diet with around 10% success from egg to adult. Most of the adults successfully emerging at the first generation from the diet were smaller, appeared weaker, and were often malformed compared to the adults emerging from the pupae collected in the wild. Morphological alterations are often a direct consequence of diet contamination, while reducing development demonstrates that the diet is missing essential components to provide the CPB's nutritional needs. Adults were fertile, and sometimes a second generation can sustain until the adult stage. This research project aims to improve the artificial diet that would allow complete development of CPB for laboratory rearing, from egg-adult to egg. The development of an optimal diet for CPB would be a significant help for further research programs and the use of sterile insect techniques.

Mass rearing of the sugar cane borer *Chilo sacchariphagus* (Lepidoptera: Crambidae) for quality SIT

BEHARY PARAY N[1] and CONLONG D E[2]

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Chilo sacchariphagus (Lepidoptera: Crambidae), commonly known as the sugar cane spotted borer, originated from South East Asia and was introduced into Mauritius in the mid- nineteenth century. It is a chronic pest of sugar cane and serious localised infestations are frequent in the dry regions. Damage can be severe in terms of loss of cane yield and sucrose content. Yield loss of 10-30 t/ha has been reported in susceptible varieties and the economic threshold level is 5 % internodes bored in Réunion and Mauritius respectively. In the eventual implementation of alternative management strategies including a SIT programme at a later stage, it is crucial to establish a cost-effective mass rearing system.

C. sacchariphagus has been reared with much difficulty on artificial diets developed for other stem borers. During the implementation of the previous CRP (IAEA D 41026), the Carcass Milling Technique was used to develop diets based upon the biochemical analyses of the larvae and the sugar cane plant parts. Though this technique was found suitable for the sugar cane borer, *Eldana saccharina* (Lepidoptera: Pyralidae) in South Africa, all the developed diets were found to be ineffective for *C. sacchariphagus*. In the present CRP (IAEA D 41028), it is intended to develop new diets based mainly upon a review of all published artificial diets for *Chilo* species. It is important to highlight that the larval feeding for *C. sacchariphagus* involves two distinct stages: the very young larvae feeding upon the young sugar cane leaves while later instars migrate to and feed inside the cane stalk. The composition of the diet will impact directly upon the quality of the insects being reared and determine how competitive they will be to the wild moths. Apart from developing cost-effective diets, there is a need to review and optimize the various rearing stages namely mating, egg production, diet seeding technology, diet enhancement through additives and automation of the rearing system including the appropriate sanitation procedures to increase productivity and reduce costs.

Developing of artificial rearing systems for cotton bollworms (Lepidoptera) in the context of Sterile Insect Technique (SIT)

Muhammad Sarwar

Pakistan Atomic Energy Commission, National Institute for Biotechnology & Genetic Engineering (NIBGE), Agricultural Biotechnology Division, Faisalabad, Pakistan

In cotton (*Gossypium hirsutum* L.) farming, bollworms are the key menace triggering an extreme produce loss, so, the goal of present research work is to establish laboratory culturing of these pests in the context of Sterile Insect Technique (SIT)**.** Adult moths feed on plant nectar, while caterpillars of these chewing worm pests move into cotton bolls, spoil bolls through eating fiber or seed and become a cause of an extreme financial damage to crop. The adults and larvae of the bollworms were collected by randomly selecting plant's leaves, buds, squares, flowers and bolls throughout the cropping season. Primarily, the collection materials were consisted of bollworms complex of cotton such as pink bollworm *Pectinophora gossypiella* (Saunders), spotted bollworm *Earias vittella* (Fabricius), spiny bollworm *Earias insulana* (Boisduval), american bollworm *Helicoverpa armigera* (Hubner), and armyworms including *Spodoptera litura* (Fabricius) and *Spodoptera frugiperda* (Smith). Based on the outcomes, the most adequate diet for rearing of all the Lepidoptera moths, was the mixture of distilled water, honey and vitamin powder based 10% solution that proved the most efficiently suitable. Studies conducted for culturing larvae of Earias, Helicoverpa and Spodoptera clearly indicated that chickpea powder, ascorbic acid, methyl 4-hydroxy benzoate, sorbic acid, streptomycin, sun flower oil, yeast, vitamin powder, agar and distilled water ingredients were equally the most adequate diet for their rearing. Neonate offspring of all these field-collected Noctuidae bollworms were individually transferred with brushes into small plastic cups having tight fitting lids and each contained nearly 5 g

of diet. After pupation, pupae from each rearing cup were collected, bulked in groups of about 25 individuals in petri dishes to permit emergence of adults, then adults shifted to rearing jars and paper stripes placed inside to serve as an oviposition substrate. Among the three modified diets for **Gelechidae** pink bollworm larva, a most suitably formulated and formalized diet based on artificial ingredients as well as cotton and okra powders as components from natural hosts, considerably produced faster population compared with the other diets. One neonate larva was placed into diet filled 24-well culture plate having the tops affixed with X-ray sheet (radiographic film) tightened with rubber bands. Afterward, pupae from each culture plate were collected and bulked in groups of about 25 individuals in petri dishes to permit emergence of adults. After pupation, upon adults emergence, moths were released in glass cages/ plastic jars for eggs laying. For getting the eggs of moth, on lid of glass/ plastic jars, put a piece of sieve, then placed tissue paper, and finally, put a small sandbag on the top of paper in order to make the paper close contact with the lid. Eggs obtained over tissue paper were collected at regular intervals and held in parafilm sealed petri dishes until they hatched, and neonates then used to infest diet for the next generation. For all bollworms, neonates to pupae developments were conducted in the dark, while all other stages (eggs and adults) maintained on a 16 h photo phase. Throughout experimentation, all life stages of bollworms were maintained at 28±1℃ and 40±10% R. H., environment.

Advancing the application of the Sterile Insect Technique (SIT) by examining the impact of some processes on the management of sugarcane borer *Eldana Saccharina* in South Africa

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The sugarcane borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is indigenous to South Africa. The stalk borer is a major limiting factor in South African sugarcane production. Several sterile moth programmes have been developed with the goal of over-flooding the natural population, but it is also crucial that the mass-reared insects can successfully compete for mating opportunities. Almost 20 years ago, South African Sugarcane Research Institute (SASRI), in conjunction with FAO and IAEA, initiated sterile insect technique (SIT) research on eldana. In the absence of an irradiator within the institute, SASRI is pioneering a novel approach to SIT. Instead of irradiating moths and releasing these directly, as in traditional SIT, the first-generation offspring are mass reared. To achieve this, eldana pupae are transported over 1000 km to XSIT, a commercial SIT facility at Citrusdal in the Western Cape, for irradiation. Male moths are partially sterilized by irradiation, mated with unirradiated females, and eggs are returned to SASRI for mass rearing and release. Full sterility is inherited in this mass reared F1 generation. Significant progress has been made to understand the factors and variables that affect the quality and performance of sterile eldana moths. Currently, research is being conducted to determine the effectiveness of SIT under controlled environment. Weekly releases of sterile male moths over the last year have significantly and continuously reduced eldana damage in sugarcane stalks and decreased the eldana population, compared to the control cage where no sterile insects were released. Other valuable lessons learned from the SIT research include the development of diet, mass-rearing of high-quality eldana moths, determination of best irradiation level, transportation and storage of pupae and eggs, as well as pilot cage releases. These promising results have encouraged a pilot field release programme, using the same principles followed under controlled environment, which is a natural progression to a full SIT programme. However, there are some aspects of the programme that needs to be addressed for a successful mass-rearing of eldana to meet the population demand for the field release. This has necessitated research into examining the impact of some processes on the management of eldana. The aim of this study is to advance the application of SIT for the control of eldana on sugarcane by investigating the effect of certain critical stages in the process. This includes (i) determining a suitable oviposition substrate, (ii) the effect of temperature on male to female emergence ratio, (iii) developing a mechanical moth collecting method, (iv) assessing the effect of radiation on egg sterility, (v) developing a mechanism to release pupae in the field and (vi) assessing the impact of handling and

transportation of moths for SIT field release. Through these experiments, protocols will be developed for mass-rearing of sterile male pupae and moths to conduct pilot field releases.

Assessing the use of cyclone separators for the improved collection of mass-reared false codling moth adults post eclosion

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The XSIT facility in Citrusdal, Western Cape is responsible of the delivery of an SIT program for the control of *Thaumatotibia leucotreta,* also called false codling moth (FCM), an important phytosanitary pest in South African citrus. The FCM eclosion facility at XSIT has a moth collection process that has been used for many years to collect moths as they eclose. However, the current system is outdated and flawed, particularly with regards to the timely collection of freshly eclosed, unmated moths for irradiation. The eclosion cabinets have been modified, using fans and UV lights, to improve the movement of moths into the canopies of the cold holding room, where the moths are stored prior to irradiation. Although these modifications increase moth collection time, the system is sub-optimal and the speed at which moths are collected could be enhanced. Furthermore, the system lacks a fitness selection process, which would improve the quality and colony fitness of the SIT moths. Additional challenges include temperature management in the cold holding rooms. The current method of moth collection causes condensation at the ideal low temperatures, due to warm air entering collection canopies from the eclosion cabinets. To optimize the timely collection of newly eclosed moths, increase moth quality and ensure correct holding temperatures are maintained, we propose that a cyclone separator be installed at the XSIT facility. The cyclone separator at the XSIT eclosion facility will be installed according to the methods used in similar mass-rearing programs. These are based off dust collection systems, which have been adapted for the collection of adult insects post eclosion. Initial research into the use of a cyclone separator at the XSIT facility has indicated that the system will be suitable for the collection of mass-reared FCM. The design and installation of the cyclone separators will be discussed, as well as experimental plans to determine the parameters required for successful FCM moth collection and for the testing of moth quality and reproductive fitness in comparison to the existing FCM moth collection process at the XSIT eclosion facility.

Develop mass rearing of the macadamia nut borer *Thaumatotibia batrachopa* (Meyrick) (Lepidoptera: Tortricidae) for quality SIT

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Thaumatotibia batrachopa (Meyrick) (Lepidoptera: Tortricidae), commonly known as the macadamia nut borer (MNB), is a significant indigenous pest of macadamia in South Africa. Nut borers generally lay their eggs on the macadamia husk, into which the larvae bore after hatching, causing direct and indirect losses to growers. Of the nut borer complex, MNB is most prevalent in commercial orchards. Feeding damage by MNB causes immature nuts to drop, secondary infections such as husk rot, and more quality defects. Commercially, it is critical to implement integrated pest management (IPM) approaches against this pest.

Rearing the pest on the natural host plant is time-consuming. In addition, frequent manipulation results in high mortality due to microbial infection. Therefore artificial diets, which meet the pest's nutritional requirements, are needed, especially if the insect is required in large numbers. Although MNB has been reared on artificial diets, the eggs laid by females have been infertile. There is thus a need, in addition to testing different diet formulations and a minimum specifications diet, to investigate the entire rearing operation to maximise the rearing efficiency of MNB.

Incorporating vegetable oils, powders and Selenium as enhancing additives to improve *Lobesia botrana* (Lepidoptera: Tortricidae) larval diet

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Lobesia botrana should be regarded as a potentially serious pest on a worldwide scale for all the vine-growing areas that are presently unaffected. The sterile insect technique/inherited sterility (SIT/IS) has been proposed as a potential control tactic for use in area-wide integrated pest management (AW-IPM) approaches against this pest. The quality of mass-reared colony insects is of primary importance to programs using the SIT/IS. A scientific literature review showed that there is a lack of specific knowledge about the effects of diet enhancing additives on the quality of *L. botrana* mass-reared moths. Therefore, the overall objective of the proposed research is to improve *L. botrana* larval diet, by supplementing the diet with certain enhancing additives, in order to increase larval and pupal yields and produce high quality moths for field release. The enhancing additives that will be used as diet supplements are: 1) ginger oil, 2) cotton seed oil, 3) grape seed powder, 4) turmeric powder, 5) cacao powder and Selenium (as chemical element). In addition, benzoic acid used in *L. botrana* artificial diet as preservative will be replaced or used in combination with the lactic acid. *L. botrana* biological characteristics that will be studied are larval and pupal developmental time, pupal weight, adult emergence, sex ratio, fecundity, adult survival, and adult flight ability. Finally, the competitiveness, mating ability, fecundity and fertility of 150 Gy-irradiated *L. botrana* male and female moths produced from the best-improved diet will be determined.

Key Objectives to Maximize Efficiency of Mass-Rearing Navel Orangeworm

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From 1994 through 2018 the Phoenix Rearing Facility (PRF) mass-reared pink bollworm (PBW) to support the sterile insect technique (SIT) component of the international PBW eradication program. By 2018, PBW had been declared eradicated throughout the commercial cotton growing regions of the US and Mexico, and so the need to mass rear PBW no longer existed. Around the same time the California Nut Industry was seeking new tools to add to their pest management options for navel orangeworm (NOW). In 2016 a project was initiated to develop mass rearing technology toward developing the SIT as a possible tool against NOW. Using the PBW equipment and procedures as a baseline, the PRF started increasing the number of NOW reared at the facility in 2017. Since then, the facility has expanded to 1.5 million moths per day and added transportation and aerial release as a demonstration project.

Among the things needed for mass production are synthetic diet, rearing containers, mass egg production and harvest techniques. Expansion of NOW mass-rearing technology is a major focus of the PRF. Recently, the PRF initiated a strain selection process intended to improve the cold tolerance of the insects. This strain has been assessed for flight initiation, response to females in field cages, wing

deformities through the collection system, susceptibility to *Bacillus thuringiensis* (Bt), egg production and overall production. Comparisons to the production strain versus the cold-selected strain (MC strain), has shown that the MC strain has improved flight ability after cold exposure, and has a lower instance of Bt. Strain selection is one approach in combating Bt in the PRF NOW colony. Multiple approaches are being evaluated to contend with the impact of Bt. A reliable detection method has been established which has led to changes in sanitation and helped identify where Bt persists in the facility. Currently there are several disinfectants being tested to kill Bt, and other control methods are being explored. Efforts to have a multi-pronged approach to dealing with Bt will continue alongside development of new rearing procedures. In particular, partial automation of the egg implant process is being tested using a soluble egg pad. This will allow eggs to be measured volumetrically and implanted on the diet using a peristaltic pump. The goal is to address a bottleneck in the system. These and other developmental work will be engaged in the coming 5 years.

Modifications in Rearing and Transport of Navel Orangeworm for Improved Effectiveness of Sterile Insect Technique.

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The navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) (NOW), is a key pest of almond, pistachio, and walnuts; California crops that are planted on >800,000 ha and worth >US$9 billion/year unprocessed. Following the successful eradication of the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidade), the lepidopteran mass rearing facility in Phoenix, Arizona has been converted to mass production and irradiation of NOW to support sterile insect technique (SIT) as a component of an area-wide program of integrated pest management (IPM). A sterilizing dose was determined (300 Gy), and capacity was developed to produce and deliver up to 2 million sterile NOW/day through a cold-chain transport system for release by fixed-wing crewed aircraft from magazines containing ~750,000 mixed sex adults. Release on > 1,000 ha of pistachios and almonds has been sustained over several years, but it has been difficult to maintain consistent overflooding ratios. Laboratory and field studies determined that male performance and recapture was not affected by irradiation at 300 Gy vs no irradiation, but it was impacted by conditions of collection and cold chain transport from Arizona to California. NOW has also proven to be particularly susceptible to mortality from sporadic outbreaks of *Bacillus thuringiensis* (Bt). Current research and methods development efforts include acclimation with cold and/or modified atmospheres for improving tolerance of transport conditions, development of pupal irradiation and F1 sterility, and reduction of Bt losses as part of overall ongoing improvement in diet, rearing, and collection procedures. Unlike some Lepidoptera, NOW males approach high concentrations of sex pheromone such has those used in the field for mating disruption. Therefore laboratory studies are also examining use of sex pheromone to separate sexes and obtain a male-enriched or male-only release. Expected results include improved efficiency of moth utilization with the given infrastructure, better inputs when using mass-released moths for models to predict impacts of pest management tactics, and ultimately more effective use of sterile NOW as part of an area-wide IPM program.

Study on artificial larval diet for mass rearing *Opisina arenosella* Walker

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Opisina arenosella Walker (common name: Black Head Catapilar) was recorded to pose severe damage on Aceraceae family trees and the damaged area could reach up to thousands of hectares in many countries all over the world. In July 2020, *Opisina arenosella* was found to appear only in a town of Ben Tre province (Viet Nam); however, it was only 8 months after that this species widely spreaded not only to the neighboring towns within Ben Tre but also to other Southern provinces. *Opisina arenosella* larva caused harm to every part of the coconut tree, including leaves and fruit, to wither, to fall and even die whether trees are at their first stage or already mature. In Vietnam, *Opisina arenosella* are being reared by leaves of coconut (*Cocos nucifera)*, Jack fruit (*Artocarpus heterophyllus*), Oil palm tree (*Elaeis guineensis*), Banana (*Musa paradise*), and White areca (*Normanbya merrillii*). As a result, it could not establish a suitable mass rearing method. Our research on artificial larvae diet with some replacement in ingredients such as soybeans powder, green bean powder and multi-vitamin is being processed by building upon previous studies. Those researches showed that *Opisina arenosella* larval was reared successfully in laboratory using a larval diet of 12 components including wheat germ, sucrose, corn meal, brewer's yeast, coconut frond powder, Wesson's salt, cholesterol, choline chloride, inositol, sorbic acid, ascorbic acid, agar and sterile water. The proposed contents of the project include: (1) Studying a simply artificial larval diet to rear BHC; (2) Development of rearing technology of BHC in small numbers. We anticipate that this research can introduce a simplified diet that is suitable for mass rearing *Opisina arenosella*, which eventually supports to biological control programmes including SIT.

ANNEX 5: MAIN HOSTS OF WORKING SPECIES

Insect species	Contractor name	Location	Diet	Host		
Conopomorpha cramerella	Savantil Anisah	Malaysia	Latest CPB diet	Theobroma cacao/pulp unripe	Theobroma cacao/pulp ripe	
	Arni Ekayanti	Indonesia	Latest CPB diet	Longan *Dimocarpus longan*	Langsat *Lansium parasiticum*	Rambutan *Nephelium lappaceum*
Lobesia botrana	George Saour	Syria	2005 Morau die	Grape/inflorescence	Grape/fruit	Olive/flower
	Nilo Oueyt	Argentina	Iscamen diet SAG chile diet	Grape/inflorescence	Grape/fruit	
	Susana Izquierdo	Chile	Lob12345	Grape/fruit	Grape/inflorescence	Blueberry/fruit
Amyelois transitella	Charles Burks	USA	ARS Bran diet ARS Agar diet	Almond/various stages of maturity	Pistachio/various stages of maturity	Walnut/various stages of maturity
	Eoin Davis	USA	USDA Standard SAS diet USDA PHY20 diet USDA CAS20 diet	Pistachio/immature nut	Almond/immature nut	
Helicoverpa armigera	Shiva Osouli	Iran	*Helicoverpa* latest diet	Tomato/fruit unripe	Tomato/fruit ripe	Pistachio/fruit unripe
	Muhammad Sarwar	Pakistan	Latest Muhammad diet	Cotton/leaves	Cotton/flower	Cotton/boll
Spodoptera litura	Muhammad Sarwar	Pakistan	Latest Muhammad diet	Cauliflower/leaves	Chickpea/leaves	Cabbage/leaves
	Hancheng Wang	China	Latest artificial diet	Tobacco/leaves	Pepper/leaves	Eggplant/leaves
Thaumatotibia leucotreta	Megan Mulcahy	South Africa	FCM diet	Citrus/ripe fruit	Plum/ripe fruit	Grape/ripe fruits
Thaumatotibia batrachopa	Stefan Foord	South Africa	Modified FCM diet FCM diet	Macademia/pericarp		
Eldana saccharina	Lawrence Malinga	South Africa	Latest diet	Sugarcane/stalk	Cyperus papyrus/inflorescence	
Opisina arenosella	Nguyen Hien	Viet Nam	Most suitable diet	Coconut/leaves	Coconut/fruit	Jackfruit/leaves
Carposina sasakii	Maohua Chen	China	Diet for the oriental fruit moth by Yu et al. (2017)	Apple/fruit	Peach/fruit	Pear/fruit
Grapholita molesta	Xiaoxia Liu	China	Oriental fruit moth diet in Yu et al. (2017)	Apple/fruit	Peach fruit/young shoot	Pear/fruit
Cydia pomonella	Evan Esch	Canada	Brinton diet	Apple/seed/pulp	Pear	walnut
Chilo sacchariphagus	Nalini Behary Paray	Mauritius	Fournier diet IAAP (CMT) diet	Sugarcane leaves *Saccharum sp.*	Sugarcane shoots *Saccharum sp.*	