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WORKING MATERIAL

Integration of SIT with Biocontrol for Greenhouse Insect Pest Management

Report of the First Research Coordination Meeting of an FAO/IAEA Coordinated Research Project

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BACKGROUND

Greenhouses and other confined locations provide ideal conditions for the rapid build-up of insect pest populations as they are largely protected from predators and parasitoids. Many of these pests have been exposed to high insecticide pressure over many generations and resistance has developed in many of them. Biocontrol agents are widely used to combat these pests, but not all are well controlled with biocontrol agents and when a pest gets out of control it has to be controlled with pesticides, which then disrupts other biocontrol and pollination.

The SIT is compatible with biological control and can complement biocontrol for those pests that are otherwise difficult to control, reducing crop losses, pesticide residues in food and risk to workers. Augmentative biological control has historically focused mainly on crops grown in confined areas. Recently there is more attention for crops grown outside. For SIT the opposite direction can be observed: historically SIT has focused on area wide pest management, but with this CRP SIT will now enter confined areas such as greenhouses.

Drosophila suzukii

Drosophila suzukii (Diptera: Drosophilidae) is an exotic pest of stone fruits and berries that has recently invaded Europe (Italy, France, Belgium, Austria, etc.), North America (United States and Canada) and South America (Brazil). This species now has a worldwide distribution (Cini et al. 2012; Asplen et al. 2015). This pest attacks a wide range of soft fruits with preference for blueberry, strawberry and raspberry (Bellamy et al. 2013), crops that can be grown in confined cropped systems. The female flies lay eggs under the skin of maturing fruits and the developing larvae feed on the fruit tissues thereby causing the fruit to collapse.

This pest is of economic importance because when left uncontrolled the flies can cause complete loss of the harvests. Currently, the control relies mostly on the application of chemical insecticides that need to be applied a few days before the fruits are harvested and may cause a threat for the health of human consumers. In addition specific cultural practices such as mass trapping, netting and strict hygiene are being used. Research on natural enemies (predators and parasitoids) is ongoing, but no biological control solutions are readily available (Cuthbertson et al. 2014; Asplen et al. 2015; Renkema et al. 2015; Stacconi et al. 2015).

Radiation biology experiments are ongoing on *D. suzukii* in collaboration with FAO/IAEA, and several universities and research institutes. Artificial rearing diets for laboratory rearing are available in the literature (Chabert et al. 2013) and at least two laboratories are conducting research on mass rearing under the Suzukill project, which is a multidisciplinary and international research project funded by both the French ANR and the Austrian FWF (https://suzukill.univ-rennes1.fr/). In addition, FAO/IAEA has had recurrent requests from member countries about developing conventional SIT for *D. suzukii*.

***Spodoptera* and *Helicoverpa* group**

Spodoptera exigua, *S. frugiperda* and *Helicoverpa armigera* (Lepidoptera: Noctuidae) share a similar biology. All three species are known as pest of both outdoor crops and of important greenhouse crops such as tomato, peppers and eggplant. Biocontrol of these species, relying on egg-parasitoids such as *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) or *Telenomus* sp. (Hymenoptera: Scelionidae), are often insufficiently effective because of the short timespan to parasitize the eggs (Jarjees & Merritt, 2004). Also, the commercially available *Bacillus thuringiensis* strains appear to be insufficiently effective (Moar et al 1995; Polanczyk et al 2000; Omoto et al 2015).

For each of these species, SIT for area wide pest management has been developed in the past (Debolt & Wright, 1976; Ocampo, 2001; Carpenter et al., 1983, 1985, 1986, 1992; Hamm & Carpenter 1997; Pransopon et al 2000). However, these were never operationalized.

Because of the past work on SIT, data on the rearing of these species and the radiation biology is available (Snow et al 1970; Snow et al 1972; Carpenter et al., 1997; Ramos Ocampo & Leon 2002; Merkx-Jacques & Bede 2005; Abbasi et al 2007). This will allow the research to quickly focus on demonstrating efficiency in greenhouses. Because the SIT for Lepidoptera normally relies on F1 sterility, a certain degree of damage needs to be tolerated. For fruit crops this tolerance is expected as the caterpillars primarily feed on the leaves, not on the fruits. On the other hand, the F1 sterility will result in increased numbers of sterile eggs in the crop. These eggs will improve the efficiency of egg parasitoids if these were to be combined with the SIT. If crop damage is not tolerable, full sterility can be considered but the high doses necessary reduce the efficacy of the control.

Because of the similarities in the biology of these three species, a CRP that coordinates the research and allows for exchange of the results is expected to lead to strong synergisms.

***Tuta* and *Neoleucinodes* group**

Tuta absoluta (tomato leaf miner) (Lepidoptera: Gelechiidae) and *Neoleucinodes elegantalis* (eggplant stem borer or tomato borer) (Lepidoptera: Crambidae) are two emerging pests of Solanaceous crops of South-American origin (EPPO, 2005). *Tuta absoluta* has currently spread eastward through Europe as far as India and northward up to Mexico (Desneux et al. 2010). Following its introduction into Europe, North Africa and the Middle East, *T. absoluta* has already caused extensive economic damage (Tropea Garzia et al. 2012). The impact of the pest includes severe yield loss reaching 100%, increasing tomato prices, bans on the trade of tomato including seedlings, an increase in synthetic insecticide applications, disruption of integrated management programmes of other tomato pests, and an increase in the cost of crop protection. Considering its high biotic potential, its ability to adapt to various climatic conditions and the speed with which it has colonized Europe and North Africa, the potential invasion of African and especially Asian tomato crops by *T. absoluta* will probably impact heavily on the livelihood of local tomato growers and tomato agribusinesses in these

regions (BBC 2016). *Tuta absoluta* in Europe is currently sufficiently controlled by the predatory mirid bugs *Nesidiocorus tenuis* and *Macrolophus pygmeus* (Heteroptera: Miridae) (Molla et al. 2009; Urbaneja et al. 2009) but these invertebrate biocontrol agents, native to Europe, will not be a control option when the pest reaches North America or Asia, which are outside of the natural enemies' native ranges. Control of the pest in South America is currently based largely on chemical control. Therefore, development of SIT for *T. absoluta* could provide a sustainable alternative. Radiation biology data for *T. absoluta* suggest doses of 200-250 Gy could be used to induce inherited sterility in *T. absoluta* males (Cagnotti et al. 2012).

Neoleucinodes elegantalis (tomato borer or eggplant stem borer) is a major pest of tomatoes and other Solanaceous fruit crops (e.g. *Solanum melongena* and *Capsicum* sp.) in South and Central America (Diaz Montilla et al. 2013). *N. elegantalis* is absent from other regions, but is considered a threat due to the importance of tomato and other Solanaceous fruit crops in many other regions. It has been intercepted several times by the Netherlands (1 interception in 2009 and 3 in 2012) during import inspections of eggplant from Suriname and control of passenger baggage at Schiphol airport. Consequently, it has been added to the EPPO Alert List.

The objective of developing a SIT program for *T. absoluta* and *N. elegantalis* is two-fold: firstly providing a more sustainable control method for currently invaded areas where biocontrol is not yet developed and secondly to provide an eradication method for these Solanaceous pests in the event they invade new areas.

CO-ORDINATED RESEARCH PROJECT (CRP)

This Coordinated Research Project (CRP) is based on a Consultants' Meeting that was held from 14-18 March 2016 in Vienna, Austria (report available) to assess the potential for conducting co-ordinated R&D in larval and adult insect for releases, and to formulate a proposal for a CRP on Integration of the SIT with control methods for greenhouse insect pest management.

The overall objective of this new CRP D43003, approved for the period 2017-2022, is to advance development and implementation of SIT for integration with other control methods in greenhouses

FIRST RESEARCH CO-ORDINATION MEETING (RCM)

Thirteen scientists from 12 countries attended this first RCM, held in Vienna, Austria from 3-7 July 2017. The list of participants, which included CRP contract and agreement holders, as well as 4 additional observers and 1 consultant, 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 (Table 1) and to review the general and specific R&D objectives to be addressed during the 5 years of the CRP and the CRP Logical Framework, in order to agree on minimum outputs to be achieved at the end of the CRP. Furthermore, participants were divided into 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 presented in Annex 4 and a copy of all PowerPoint presentations was made available to all participants at the end of the RCM.

Table 1. Thematic areas in relation to pest species being addressed by researchers.

Species	Greenhouse trials	Mass rearing	Radiation effects	Integration with biocontrol	Biology	Standardized protocols
Drosophila suzukii		Aikaterini Nikolouli Massimo Cristofaro Gustavo Taret Fabianna Sassu	Massimo Cristofaro Anna Malacrida Zhu Zeng-Rong Fabiana Sassu Gustavo Taret Alexandra Krüger Victor Gutierrez Annabelle Firlej Aikaterini Nikolouli	Alexandra Krüger Massimo Cristofaro Victor Gutierrez Gustavo Taret Zhu Zeng-Rong	Anna Malacrida Allan Debelle Massimo Cristofaro Gustavo Taret Alexandra Krüger Zhu Zeng-Rong	Massimo Cristofaro Anna Malacrida Zhu Zeng-Rong Fabiana Sassu Gustavo Taret Alexandra Krüger Victor Gutierrez Annabelle Firlej Aikaterini Nikolouli
Tuta absoluta	Carolina Yanez Waheed Sayed	Carolina Yanez Waheed Sayed V.P. Venugopalan	Carolina Yanez Waheed Sayed V. P. Venugopalan	Carolina Yanez Waheed Sayed V. P. Venugopalan		
Spodoptera littoralis	Waheed Sayed	Waheed Sayed	Waheed Sayed	Waheed Sayed		
Heliocoverpa armígera	Muhammad Zahid	Muhammad Zahid	Muhammad Zahid	Muhammad Zahid		

1. STERILE INSECT TECHNIQUE FOR *D. SUZUKII*

Background Situation Analysis

Drosophila suzukii (Diptera: Drosophilidae) is a pest of East Asian origin. This pest attacks a wide range of stone fruits and berries of economic importance with preference for blueberry, strawberry and raspberry (Bellamy et al. 2013), crops that can be grown in confined systems. It started to invade Europe (Italy, France, Belgium, Austria, etc), North America (United States) in 2008 and South America in 2013 (Brazil). This species now has a worldwide distribution (Cini et al. 2012; Asplen et al. 2015). Unlike other *Drosophila* species, *D. suzukii* females lay eggs under the skin of maturing fruits and the developing larvae feed on the fruit tissues, thereby provoking the fruit to collapse. The females can lay eggs in fruits close to harvest and also before. This pest is very adaptable to different environments, host plants and abiotic conditions (temperature, humidity, etc).

Drosophila suzukii is a quarantine pest for New Zealand and Australia. This pest is of economic importance because, when left uncontrolled, it can cause complete harvest loss. Currently the control relies mostly on the application of chemical insecticides that need to be applied a few days before the fruits are harvested, which may cause a threat to consumers' health. In addition, specific practices such as mass trapping, netting and strict sanitation are being used. Research on natural enemies (predators and parasitoids) is ongoing, but no biological control solutions are readily available (Cuthbertson et al. 2014; Asplen et al. 2015; Renkema et al. 2015; Stacconi et al. 2015).

***Drosophila suzukii* biology**

Current knowledge:

This pest has a high reproductive potential. The generation time of this multivoltine species is dependent on several factors (climate conditions, crops, etc.), and it has been reported that the adult fly can survive as a winter morph that undergoes reproductive diapause (Stacconi et al. 2016; Toxopeus et al. 2016). Stacconi et al. (2016) suggest that most overwintering adults are females. When females lay eggs in fruits, the oviposition scars could be a door for pathogens (e.g. bacterial and fungal infections), an important point to consider for application of SIT in the absence of a sexing system (i.e. release of both sexes).

Concerning reproduction and mating behavior, there is little knowledge. The male can mate (upon emergence and female at least after 12 h. *Drosophila suzukii* uses visual (black dots and wing display; Revadi et al. 2015, Fuyama 1979) and vibrational courtship signals ("toots" and abdomen quivering; Revadi et al. 2015, Mazzoni et al. 2013). It is unclear whether *D. suzukii* is using chemical signals for mating, and their role must be defined (Dekker et al. 2015; Revadi et al. 2015).

Gaps identified:

1. Lack of characterisation of populations from different origins in terms of: genetic variability, pre-zygotic and post-zygotic compatibility.
2. Lack of knowledge about which male sexual traits (morphological, acoustic and chemical) are preferred by females, as well as which male traits are involved with increased competitiveness.
3. The level of *D. suzukii* polyandry is unclear because of confusing findings and should be investigated.
4. Sexual maturity of females and males (receptivity, fertility and pre-oviposition period) should be physiologically studied.
5. Influence of the maturity of the fruits on oviposition preference and progeny fitness (larval development, adult production, etc) should be investigated.
6. Factors that could influence oviposition choice in term of marking pheromome or plant kairomones are unknown.
7. The dynamics of *D. suzukii* populations in greenhouses (sex-ratio, circadian cycle, seasonality…) are not defined.

Radiation biology for *D. suzukii*

Current knowledge:

The dose-response for males and females at the pupal stage in the presence or absence of O_2 (i.e. normoxia/hypoxia) is under evaluation (Lanouette et al. accepted; Sassu et al. unpublished data, Krüger et al. unpublished data). First experiments show that the dose-response for female pupal sterility lies between 30-50 Gy, depending on atmospheric conditions (Lanouette et al. accepted; Sassu et al. unpublished data). Sassu et al. are using a range from 30 to over 200 Gy, measuring several sterility parameters (i.e. egg hatching, pupae recovery, adult survival).

Competitiveness experiments of irradiated individuals have been performed in small laboratory cages for doses ranging from 80 to 120 Gy (Firlej et al. unpublished data). All experiments were made with cesium or cobalt irradiation.

Gaps identified:

1. The response for irradiation doses above 120 Gy should be completed in order to determine the doses that induce full sterility. Quality control test (longevity, weight of pupae, survival, flight…) should be performed for higher doses than 120Gy, to determine the balance between sterility induction and sterile male fitness. It is crucial to do the characterisation of the sexual competitiveness of irradiated males (mating, flight, dispersion capacity, etc) to select the appropriate doses inducing sterility.
2. Information on sperm viability and sperm competitiveness of irradiated males (morphology, ejaculate size, sperm number, etc) is lacking, as well as the existence of cryptic female choice between irradiated and non-irradiated sperm.

3. Effect of irradiation doses on sperm/ejaculate transfer, refractory period and re-mating frequency is important and unknown.
4. The best time interval for egg collection, as well as the optimal female age, should be evaluated to ensure optimal progeny collection (progeny quality, irradiation effectiveness and genetic variability).
5. Mating compatibility within a strain between irradiated laboratory males and wild females (colonization effect) is unknown, as well as the mating compatibility between strains of different origins.
6. The effects of sterile female oviposition (i.e. skin puncturing) on fruit quality and susceptibility to pathogenic infections have to be evaluated.
7. Impact of hypoxia and temperature before and after irradiation on quality control parameters should be evaluated.

Potential sexing system

Current knowledge:

The IAEA is running a CRP (D42016) on genetic sexing system for different species, including *D. suzukii*. The team of Marc Schetelig (Germany) is developing a sexing system based on CRISPR/Cas9. Other teams are working on genetic sexing systems in the USA and UK.

Gaps identified:

1. Genetic sexing systems based on pupal characteristics or embryonic lethality should be developed.

Mass rearing for *D. suzukii*

Current knowledge:

Different artificial diets are used for *D. suzukii* rearing (Chabert et al. 2012, Lanouette et al. accepted), but optimization should be achieved. Influence of temperature and humidity were measured on *D. suzukii* egg, larval and adult survival and development, and the optimal temperature was determined (Gutierrez et al. 2015). The tolerance of *D. suzukii* pupae to cold storage is currently under evaluation by the SUZUKILL project.

Gaps identified:

1. Synchronisation of pupae emergence should be evaluated to improve the quality of the mass rearing and its cost-effectiveness.
2. The definition of optimal mass-rearing artificial diet(s) for high quality larval and adult performance is lacking.
3. Techniques for egg collection and pupal separation are lacking.
4. Cost analysis of mass-rearing is missing.

Integration of SIT with other method of control for *D. suzukii*

Current knowledge:

The efficacy of other control methods for *D. suzukii* are currently being evaluated, such as the use of 1) parasitoids/predators (Daane et al. 2016; Renkema et al. 2015), 2) fungi, bacteria and nematods (Cossentine et al. 2016, Cuthberstson et al. 2014; Naranjo-Lazaro et al. 2014); 3) mass trapping (Baroffio, Bolta INTA), 4) chemicals (Beers et al.), and 5) net exclusion (Cormier et al. 2015; Grassi et al. 2016).

Gaps identified:

1- The evaluation of the compatibility of these other control methods with SIT is missing.

1.1. Standard protocols

Participants: all participants

5 year plan:

- Create a protocol for all teams to perform standardised experiments: traps and baits used for sampling, artificial diet for rearing, dosimetry, voucher sampling frequency.

18 month plan:

- Create a protocol for all teams to perform standardised experiments: traps and baits used for sampling, artificial diet for rearing, dosimetry, voucher sampling frequency.

1.2. Drosophila suzukii biology

Participants: Anna Malacrida (Italy)

5 year plan:

- Characterisation of strains in terms of genetic background (in collaboration with all the partners)
- Evaluation of the polyandry level at different population densities in different greenhouse types (in collaboration with all the partners).
- Evaluation of the polyandry level and sperm use in different strains, populations from different geographical origins (in collaboration with all the partners)
- Define different molecular marking systems for strain differentiation (in collaboration with all the partners)
- Evaluate the impact of different larval diets on the production of male and female sexual chemical signals (in collaboration with IPCL, Allan Debelle and Gustavo Taret)

18 month plan:

- Characterisation of strains in terms of genetic background (in collaboration with all the partners)

- Evaluation of the polyandry level and sperm use in different strains, populations from different geographical origins (in collaboration with all the partners)

Participants: Allan Debelle (France)

5 year plan:

- Identify the traits involved in male mating success variation in *D. suzukii* (both for male attractiveness and male competitiveness) (In collaboration with Anna Malacrida for chemical signals)
- Estimate the optimal values of these traits for maximising male mating success (both for male attractiveness and male competitiveness) (In collaboration with Anna Malacrida for chemical signals)
- Analyse the social network structure in *D. suzukii* at the group level and identify advantageous male phenotypes for an SIT approach (e.g. how socially connected a male is)
- Assess the potential for transferability of these traits (genetic basis)
- Compare the effects of these traits between artificial and natural conditions (In collaboration with Annabelle Firlej)

18 month plan:

- Identify the traits involved in male mating success variation in *D. suzukii* (both for male attractiveness and male competitiveness) (In collaboration with Anna Malacrida for chemical signals).
- Estimate the optimal values of these traits for maximising male mating success (both for male attractiveness and male competitiveness) (In collaboration with Anna Malacrida for chemical signals).

Participants: Massimo Cristofaro (Italy)

5 year plan:

- Develop a suitable standard monitoring system to quantify population density (e.g. sex-ratio) in greenhouse conditions (In collaboration with Alexandra Krüger).
- Verify the reproductive physiological status of newly emerged females (i.e. already mated or not), by using morphological and genetic tools (In collaboration with IPCL and Anna Malacrida)
- Carry out laboratory tests to evaluate the presence of species-specific mechanisms of previous oviposition recognition (repellence/attraction), by using behavioral and olfactory studies.

18 month plan:

- Develop a suitable standard monitoring system to quantify population density (e.g. sex-ratio) in greenhouse conditions (In collaboration with Alexandra Krüger).

- Verify the reproductive physiological status of newly emerged females (i.e. already mated or not), by using morphological and genetic tools (In collaboration with IPCL and Anna Malacrida)

Participants: Gustavo Taret (Argentina)

5 year plan:

- Develop GIS system to be applied on *D. suzukii* to be adapted to fruit flies and other pests in Argentina.

18 month plan:

- Develop GIS system to be applied on *D. suzukii* to be adapted to fruit flies and other pests in Argentina.

Participants: Flavio Garcia, Dori Nava and Alexandra Krüger (Brazil)

5 year plan:

- Estimate the relationship between population density and *D. suzukii* trap catches in different crops (in collaboration with Massimo Cristofaro and Victor Manuel Gutierrez)

18 month plan:

- Estimate the relationship between population density and *D. suzukii* trap catches in different crops (in collaboration with Massimo Cristofaro and Victor Manuel Gutierrez)

Participants: Zhu Zeng-Rong (China)

5 year plan:

- Collection and identification by morphological and molecular methods *D. suzukii* and its natural enemies from Zhejiang and neighbouring provinces, and establishment and characterization of laboratory populations of East Asian strains.

18 month plan:

- Collection and identification by morphological and molecular methods *D. suzukii* and its natural enemies from Zhejiang and neighbouring provinces, and establishment and characterization of laboratory populations of East Asian strains.

1.3. Drosophila suzukii radiation biology

Participants: Massimo Cristofaro (Italy)

5 year plan:

- Histological and genetic evaluation of radiation effects on sexual organs (in particular testes) and sperm viability (in collaboration with Anna Malacrida and IPCL).
- Dispersal of SIT flies in greenhouses, in comparison with unirradiated flies, with different doses of radiation and different dyeing (in collaboration with Annabelle Firlej).
- Develop a biochemical/morphological protocol to evaluate the impact of irradiated or unirradiated female oviposition on fruit quality (in collobaoration with IPCL)

18 month plan:

- No plan for the first 18 months.

Participants: Anna Malacrida (Italy)

5 year plan:

- Evaluation of the impact of irradiation on sperm use, mating and remating frequency (in collaboration with IPCL).
- Evaluation of irradiation on chemical signals, and the consequences on mating success (in collaboration with IPCL, Gustavo Taret and Allan Debelle).

18 month plan:

- Evaluation of the impact of irradiation on sperm use, mating and remating frequency (in collaboration with IPCL).

Participants: Zhu Zeng-Rong (China)

5 year plan:

- Radiation biology and quality control of East Asian *D. suzukii*.

18 month plan:

- Radiation biology and quality control of East Asian *D. suzukii*.

Participants: Fabiana Sassu and Carlos Caceres (Vienna)

5 year plan:

- Develop dose-response functions for *D. suzukii* pupae with Gamma and X ray under different atmospheric conditions.
- Competitiveness test of different radiation doses in laboratory and field cages.

18 month plan:

- Develop dose-response functions for *D. suzukii* pupae with Gamma and X ray under different atmospheric conditions.

Participants: Gustavo Taret (Argentina)

5 year plan:

- Determination of the post-irradiation quality parameters to guarantee competitive sterile males in the field.
- Determination of compatibility, competitiveness and induced sterility tests in semi-field conditions for laboratory and field strains (from different geographical regions in Argentina).
- Evaluation of the effectiveness of the use of the SIT in confined farms (greenhouses).

18 month plan:

- No plan for the first 18 months.

Participants: Flavio Garcia, Dori Nava and Alexandra Krüger (Brazil)

5 year plan:

- Evaluation of the effects of sterilisation on female remating and fertility recovery as well as on the competitiveness of sterile males at different radiation doses.
- Evaluation of the effects of abiotic factors (temperature, humidity and fungicides/acaricides) on irradiated *D. suzukii* adults (in collaboration with Annabelle Firlej and Allan Debelle).
- Identification of the best overflooding ratio and frequency of releases of sterile males in greenhouse.

18 month plan:

- Evaluation of the effects of abiotic factors (temperature, humidity and fungicides/acaricides) on irradiated *D. suzukii* adults (in collaboration with Annabelle Firlej and Allan Debelle).

Participants: Victor Gutierrez (Mexico)

5 year plan:

- Evaluation of the effects of gamma radiation on different pupal ages of *D. suzukii* (induction of sterility, quality of adults).

18 month plan:

- Evaluation of the effects of gamma radiation on different pupal ages of *D. suzukii* (induction of sterility, quality of adults).

Participants: Annabelle Firlej (Canada)

5 year plan:

- Evaluation of competitiveness at different radiation doses in field cages.
- Assessing mating compatibility between wild and laboratory strains, as well as between strains of different geographical origins (in collaboration with all participants).
- Testing the effects of different abiotic conditions on irradiated pupal survival and irradiated male mating success (In collaboration with Allan Debelle and Alexandra Krüger).
- Effet of radation doses on flight capacity and dispersal (in colloboration with Massimo Cristofaro).

18 month plan:

- Evaluation of competitiveness at different radiation doses in field cages.
- Testing the effects of different abiotic conditions on irradiated pupal survival and irradiated male mating success (in collaboration with Allan Debelle and Alexandra Krüger).

Participants: Aikaterini Nikolouli and Konstantinos Bourtzis (Vienna)

5 year plan:

- Developing a combined SIT/IIT approach for *D. suzukii*.

18 month plan:

- Fitness and cytoplasmic incompatibility (CI) experiments will be performed for all *Wolbachia* infected and uninfected lines of *D. suzukii*.
- Effect of male age on CI will be investigated.
- Competitiveness experiments will be performed in population cages. Different ratios of males infected by wHa and wTei will be tested.

1.4. Mass rearing for D. suzukii

Participants: Aikaterini Nikolouli and Konstantinos Bourtzis (Vienna)

5 year plan:

- Detecting and managing genetic changes and symbiont changes during laboratory domestication of *D. suzukii*.
- Pool-Seq will be applied in the intermediate generations, if we detect rapid genetic changes.

18 month plan:

- The wild population will be collected by October 2017.
- Biological quality tests will be performed on the F0 wild population, and then at F5, F10, F15 and F20.
- After six months, the domesticated populations will be approximately at generation 11-12. DNA will be extracted by adults and sent for Pool-Seq from the following generations: F0, F1, F2, F5 and F10.
- Gut dissections will be done in the following generations: F0-F10, F15 and F20. 16S rRNA gene clone libraries will be constructed and sent for Next-Generation Sequencing.

Participant: Massimo Cristofaro (Italy)

5 year plan:

- Performing bioassays to evaluate the most suitable age for oviposition to get the best quality eggs.

18 month plan:

- Performing bioassays to evaluate the most suitable age for oviposition to get the best quality eggs.

Participants: Gustavo Taret (Argentina)

5 year plan:

- Develop a mass-rearing system for *D. suzukii*.
- Develop a system for packaging and releasing sterile *D. suzukii* (pupae or adults).

18 month plan:

- Develop egg collection and pupal separation technique.
- Measure colonisation effects in laboratory rearing.

Participants: Fabianna Sassu and Carlos Caceres (Vienna)

5 year plan:

- Develop a mass-rearing system for *D. suzukii*.

- Evaluation of different artificial diets on larval/adult fitness.

18 month plan:

- Evaluation of different artificial diets on larval/adult fitness.

1.5. Integration of SIT with other method of control for D. suzukii

Participants: Flavio Garcia, Dori Nava and Alexandra Krüger (Brazil)

5 year plan:

- Develop the combined use of SIT and parasitoid species (i.e. *Trichopria anastrephae*).

18 month plan:

- No plan for the next 18 months.

Participants: Massimo Cristofaro (Italy)

5 year plan:

- Develop the combined use of SIT and indigenous parasitoid species.

18 month plan:

- No plan for the next 18 months.

Participants: Victor Gutierrez (Mexico)

5 year plan:

- Evaluate the combination of biological control methods based on fungi (Pf21, Pf17, Pf15 and *Metarhizium anisopliae*) with SIT.
- Evaluate the adult quality of *D. suzukii* infected by fungi (Pf21, Pf17, Pf15 and *Metarhizium anisopliae*) as a vector.

18 month plan:

- No plan for the next 18 months.

Participants: Gustavo Taret (Argentina)

5 year plan:

- Evaluation of the effectiveness of the integration of SIT integrated with other control methods (e.g. pesticides, mass trapping or cultural control).

18 month plan:

- No plan for the next 18 months.

Participants: Zeng Rong-Zhu (China)

5 year plan:

- Integration of SIT with a biological control method (i.e. parasitoid).

18 month plan:

- No plan for the next 18 months.

2. STERILE INSECT TECHNIQUE FOR LEPIDOPTERA

Background Situation Analysis

Tomato leaf miner, *Tuta absoluta* (Meyrick) is considered a major global invasive insect pests on several solanaceous crops.

Fruit worm, *Helicoverpa armigera* (Hub.) is the major insect pest of tomato and okra crops in Pakistan. All crops are under severe attack of insect pests which result in heavy losses to growers.

The cotton leaf worm, *Spodoptera littoralis* (Boisd.) is one of the most important insect pest around the world. It is a highly polyphagous defoliator of many cultivated plants with a wide range of hosts, feeding on 112 species worldwide.

2.1. Monitoring and mass rearing of Lepidoptera

Current knowledge:

Mass rearing of insects is a key component of the SIT. Lepidopteran insects are commonly reared on artificial diets to reduce the time, space and associated costs of growing their host plants. The development of these artificial diets with the availability of food are still required in many of lepidopteran insect and can be optimized to increase insect fitness.

The objectives can be summarized as follow:

1- To develop standard protocols for infestation level assessment and host range in the participating countries.
2- Colonisation of culture from field collected insects.
3- Cost and quality of larval diet (locally available ingredients).
4- Improving mass rearing and quality of the insects produced.
5- Sharing mass rearing protocols among the participants.

Participants: Yanez Carolina, Castro David, Izquierdo Susana, Pavez Viviana and Sour George (Chile and Syria)

5 year plan:

- Develop standard protocols for infestation level assessment.
- Establishing and maintaining *T. absoluta* colony in the laboratory.
- Studying the possibility of rearing *T. absoluta* on artificial diet.
- Studying the quality control parmeters of reared insect.

18 month plan:

- Establishing and maintaining *T. absoluta* colony in the laboratory
- Studying the possibility of rearing *T. absoluta* on artificial diet: The insect will start to be reared under laboratory condition in growth chambers in mesh netting cages containing potted tomato plants. Adult's moths will be fed on honey placed on the mesh inside the cages and on the plants.

Participants: Waheed Sayed, Alexandra Elhelaly, Gamal Hassan, Farha Hosny (Egypt)

5 year plan:

- Developing the artificial diet of *S. littoralis*.
- Establishing and maintaining *T. absoluta* colony in the laboratory.
- Establishment *T. absoluta* mass rearing.
- Studying the quality control parmeters of reared insect.

18 month plan:

- Developing the mass rearing of *Spodoptera littoralis* by modified the standard diet and reared the *T. absoluta* of sugested ingredient and studying the mass rearing and quality control parmeters.
- Establishing and maintaining *T. absoluta* colony in the laboratory.
- Establishing *T. absoluta* rearing on artificial diet

Participants: Ramesh Hire; Ashok Hadapad and V.P. Venugopalan (INDIA)

5 year plan:

- Collection of tomato leaf miner *Tuta absoluta* samples from different states of India using TLM lures.
- Studying the genetic diversity of tomato leaf miner populations.
- Establishment of mass rearing protocols to meet the supply and demand for SIT.

18 month plan:

- Collection of tomato leaf miner from different states (Maharashtra, Karnataka, Andhra Pradesh, Bihar, West Bengal, Tamil Nadu and Gujarat) of India using TLM lures.
- Assessment of genetic diversity of collected tomato leaf miner populations using mitochondrial gene fingerprinting techniques.

Participants (Muhammad Zahid, Muhammad Salman and Alamzeb (PAKISTAN)

5 year plan:

- Develop standard protocols for infestation level assessment of *H. armigera*
- Develop the artificial diet of *H. armigera*
- Establishing and maintaining *H. armigera* colony in the laboratory

18 month plan:

- Effect of Chilling on hatching of *S. cerealella*: Three replication of *S. cerealella* (24 hrs old-eggs; 500 eggs/card) will be chilled at -1°C for 8, 12, 24, 48 & 72 hours as compared to control. The cards will be kept in glass jars on wheat kernels till hatching.

2.2. Radiation effects

Current knowledge

Application of SIT requires an irradiation system. Currently, the available irradiators of a suitable size for research, development and small scale production include both gamma and X irradiators. Lepidopteran pests are successefully manged by gamma irradiation in many regions around the world. Inherited sterility (IS) offers significant advantages over classical sterile released method for lepidopteran pests. The IS technique depends on using substerlizing doses of gamma radiation, these doses induce deleterious effects that are inherited by the F1 generation. IS was employed in many regions to eradicate Lepidopteran pests.

The objectives can be summerized as follow:

1. Detereming the sterilizing doses of target insect pests
2. Detereming the sub-sterilizing doses of target insect pests
3. Optimisation of radiation dose for lepidopteran insect pests
4. Dose-response curve to be determined for different lepidopteran insect pests

Participants: Yanez Carolina, Castro David, Izquierdo Susana, Pavez Viviana and Sour George (Chile and Syria)

5 year plan:

- Detereming the sterilizing doses of *T. absoluta*
- Detereming the sub-sterilizing doses of *T. absoluta*

18 month plan:

- Detereming the sterilizing doses of *T. absoluta* by irradiated full-grown pupae 48 hours before emergence will irradiate by sterilizing and sub-sterilizing doses of gamma radiation using cobalt-60 located at the CCHEN (Chile)

Participants: Waheed Sayed, Alexandra Elhelaly, Gamal Hassan, Farha Hosny (Egypt)

5 year plan:

- Detereming the sterilizing doses of *T. absoluta* insect pests
- Detereming the sub-sterilizing doses of *S. littoralis* and *T. absoluta* insect pests
- Optimisation the irrradiation doses for *S. littoralis* and *T. absoluta* insect pests

18 month plan:

- Determine the sub-sterilizing doses of *S. littoralis* and *T. absoluta* insect pests.
- Full-grown pupae of test insect 24 hours before emergence will be irradiated by sterilizing and sub-sterilizing doses of gamma radiation using cobalt-60 gamma cell 3500 source located at the Cyclotron Project, Nuclear Research Centre, Egyptian Atomic Energy Authority.

Participants: Ramesh Hire; Ashok Hadapad and V. P. Venugopalan (INDIA)

5 year plan:

- Detereming the sterilizing doses of *T. absoluta*
- Detereming the sub-sterilizing doses of *T. absoluta*

18 month plan:

- No plan for the next 18 months.

Participants (Muhammad Zahid, Muhammad Salman, Alamzeb (PAKISTAN)

5 year plan:

- Detereming the sterilizing doses of *H. armigera*
- Detereming the sub-sterilizing doses of *H. armigera*

18 month plan:

- No plan for the next 18 months.

2.3. Biocontrol Methods

2.3.1. Egg parasitoid, *Trichogramma* studies: Current knowledge

Several *Trichogramma* species have been tested for their parasitizing efficiency against lepidopteran eggs. However, little information is available on the per cent *Trichogramma* adult emergence from parasitized *Tuta* eggs, and the parasitizing efficiency of the emerged adults (Ballal et al., 2016). Over 200 species of *Trichogramma* have been recorded in India. Several *Trichogramma* species are known to parasitize several lepidopteran insect eggs (e.g. *Helicoverpa*, *Spodoptera*, *Plutella*, *Chilo* sp etc) on different crops and are being used as biocontrol agent in integrated pest management (IPM) programmes.

Typically, 50 000-250 000 parasitoids/ha are being released under field conditions at weekly intervals along with other control strategies. Among the trichogrammatids, *T. chilonis* is widely used in IPM in India, China, Korea, Taiwan, Japan, Pakistan, Nepal, Reunion Island and as exotic species in Kenya, Spain, South Africa and Australia

2.3.2. Baculoviruses

Baculovirus products are commercially available under various trade names for use in certain parts of the world. *Spodoptera littoralis* nucleopolyhedro virus (SpliNPV) was successful applied in many regions. However, UV radiation and the immune systems of insects makes the use of NPV control less effective, hence additional methods are needed to achieve an adequate level of control.

2.3.3. Integration of SIT with Biocontrol Methods

To the best of our knowledge, no study has been conducted to examine the acceptability and suitability of *T. absoluta* eggs from irradiated parents to parasitism by *T. achaeae*, in contrast this parameter has been recently studied for *T. nerudai* and *T. pretiosum* by Cagnotti et al. (2016); nor the effects of combining the Inherited Sterility technique and the release of *Trichogramma* egg parasitoid (*T. achaeae*) to suppress *T. absoluta* populations in greenhouses. Therefore, the main objectives of the proposed research are the following:

Participants: Yanez Carolina, Castro David, Izquierdo Susana, Pavez Viviana and Sour George (Chile and Syria)

5 year plan:

- Studying the parasitization of *T. absoluta* eggs by *T. pretiosum*, *T. nerudai* and *T. achaeae* in choice and no-choice tests conducted under laboratory conditions.
- Evaluating the integration of SIT with *T. pretiosum*, *T. nerudai* and *T. achaeae* to control *T. absoluta* populations under tomato greenhouse environments.

18 month plan:

- No plan for the next 18 months.

Participants: Waheed Sayed, Alexandra Elhelaly, Gamal Hassan, Farha hosny (EGYPT)

5 year plan:

- Studying the parasitization of *T. absoluta* and *Spodoptera littoralis* eggs with irradiated insects (pupae).
- Studying the integration of SIT with *Spodoptera littoralis* nuclepolyhedrovirus (Spli NPV).
- Attempt to isolate *T. absoluta* viruses.

18 month plan:

- Studying the integration of SIT with *Spodoptera littoralis* nuclepolyhedrovirus (Spli NPV).

Participants: Ramesh Hire; Ashok Hadapad and V. P. Venugopalan (INDIA)

5 year plan:

- Studying the parasitization of *T. absoluta* and *Spodoptera litura* eggs with irradiated insects (pupae).

18 month plan:

- No plan for the next 18 months.

Participants (Muhammad Zahid, Muhammad Salman and Alamzeb (PAKISTAN)

5 year plan:

- Impact of gamma radiation on hatching of *S. cerealella* and also on egg parasitization by *T. chilonis*.
- Improvement and establishment in the insectary of the egg parasitoid, *Trichogramma chilonis* (Ishii) on *Sitotroga cerealella* (Oliv.).
- *Trichogramma chilonis* (Ishii) culture.
- Effect of the age of *S. cerealella* eggs on oviposition preference of *T. chilonis*.
- Parasitism of *S. cerealella* eggs as influenced by the age of *T. chilonis*.
- Oviposition preference of female *T. chilonis* on different colour cards of host eggs, *S. cerealella*.
- Impact of gamma radiation on hatching of *S. cerealella* and also on egg parasitization of *T. chilonis*.
- Effect of chilling on hatching of *S. cerealella* and on egg parasitization of *T. chilonis*.

18 month plan:

- Improvement and establishment in the insectary of the egg parasitoid, *Trichogramma chilonis* (Ishii) on *Sitotroga cerealella* (Oliv.).
- *Trichogramma chilonis* (Ishii) culture: *Trichogramma* will be reared on the eggs of *S. cerealella* in bio-control laboratory at NIFA, Peshawar. Wheat grains will be used as a rearing medium. The moths will be collected through an electrically operated suction apparatus and caged in oviposition chambers (2 L plastic jars with a wire mesh of 20 holes/sq. inch size at the bottom). Wheat starch will be used as an oviposition substrate and eggs of *S. cerealella* will be collected daily by sieving the wheat starch. Egg cards will be prepared by gluing *S. cerealella* eggs to paper cards (9×4 cm) and exposing them to *T. chilonis* in a 300 ml glass jar for 24 hours. The parasitoids will be fed with 50% honey solution provided as small drops on the walls of the glass jar. The cards will be removed from the jar and kept in petridishes under controlled conditions (23+2 0C, 60% R.H. &16 L: 8D) for use in the experiments.
- Effect of the age of *S. cerealella* eggs on oviposition preference of the female parasitoid, *T. chilonis*: *Sitotroga cerealella* eggs of 12, 24, 48 & 72 hours of age will be exposed to parasitization by *T. chilonis* females in a no-choice experiment. A prepared egg card containing approximately 500 host eggs of each age will be introduced in 300 ml glass parasitization jars containing 10 pairs (male & female) of freshly emerged less than one day old *T. chilonis* & will be removed after 24 hrs. The parasitized egg card will be maintained at 23-25°C, 16L: 8D until melanization of the parasitized eggs. The parasitized and un-parasitized eggs will be counted and the percentage of parasitism of *S. cerealella* eggs will be calculated.
- Parasitism of *S. cerealella* eggs as influenced by the age of the female parasitoid, *T. chilonis*: The effect of the age of *T. chilonis* female on the extent of parsitization of *S. cerealella* will be investigated. *T. chilonis* females of different age groups (treatments), 8-10, 16, 36, 48, 72 & 96 hours after emergence will be used in the experiment. Fresh eggs (4-6 hrs old) of *S. cerealella* will be glued on hard paper card at 500 eggs/ card. Fifty pairs (males & females) of each age group of *T. chilonis* will be introduced into each of the 300 ml of glass jar containing a prepared egg card and will be allowed to parasitize for 24 hrs. The egg card will be removed and the extent of parasitism will be noted by counting the total number of parasitized & un-parasitized eggs.
- Oviposition preference of the female parasitoid, *T. chilonis* on different colour cards of host eggs, *S. cerealella*: Eggs of *S. cerealella* will be sprinkled on six different colour cards i.e., white, red, yellow, green, blue and black for maximum parasitization. These cards will be exposed to parasitize by 10 pairs of fresh *T. chilonis* females. A prepared egg card containing 500 host eggs of each age will be introduced in a parasitization glass jar containing 50 pairs (male & female) of fresh *T. chilonis* for 24 hrs. The parasitized and un-parasitized eggs will be counted and % parasitism of *Sitotroga* eggs will be calculated on each colour card.

- Effect of chilling on egg parasitization of *T. chilonis*: Three replication of *S. cerealella* 24 hrs old egg strips with 500 eggs/strip will be chilled at -1°C for 8, 12, 24, 48 & 72 hours then exposed to 10 pairs of parasitoids, *T. chilonis* to allow parasitism for 24 hours. The parasitized eggs will be kept in glass tubes to calculate the parasitism in each treatment and effect of host eggs age will also be determined.
- Impact of gamma radiation on hatching of *S. cerealella*: Fresh eggs of *S. cerealella* will be obtained from stock culture of bio-control laboratory of NIFA, Peshawar. Three replications of 500 eggs irradiated at different doses of 10, 20, 30, 40 and 50 Gy will be placed in glass jars provided with wheat kernels as rearing media to record the % hatch.
- Effect of gamma radiation on egg parasitization of *T. chilonis*: *Sitotroga* eggs will be sprinkled on white paper cards having gum as sticking material. After one hour the cards will be exposed to radiation doses of 10, 20, 30, 40 and 50 Gy. Each card containing 500 irradiated eggs will be exposed to 10 pairs of parasite, *T. chilonis* in glass jars and to observe the effect of host egg age for 24 hrs. The % parasitism on each card and effect of age of host eggs for parasitization will be recorded and compared with untreated control and will be replicated three times.

2.4. Greenhouse Experiments

Current knowledge

The four participants will carry out greenhouse studies to implement SIT or inherted sterility integrated with biocontrol methods to control the target pest in confined cropping systems. The experiments will be conducted by releasing sterile moths of the target insect with *Trichogramma* or Baculoviruses to compare the different treatments (sterile insect and sterile insect with biocontrol methods).

Participants: Yanez Carolina, Castro David, Izquierdo Susana, Pavez Viviana and Sour George (Chile and Syria)

5 year plan:

- Integration of SIT with *Trichogramma* spp. to suppress the population of *T. absoluta*.

18 month plan:

- No plan for the next 18 months.

Participants: Waheed Sayed, Alexandra Elhelaly, Gamal Hassan and Farha hosny (EGYPT)

5 year plan:

- Integration of SIT with *Trichogramma* spp to suppress the population of *T. absoluta*.
- Integration of SIT with *Spli NPV* to suppress the population of *S. littoralis*.

18 month plan:

- No plan for the next 18 months.

Participants:

5 year plan:

- Integration of inherited sterility with *Trichogramma* spp. to suppress the population of *T. absoluta*.

18 month plan:

- No plan for the next 18 months.

Participants: Ramesh Hire, Ashok Hadapad and V. P. Venugopalan (India)

5 year plan:

- Integration of inherited sterility with *Trichogramma* spp. to suppress the population of *T. absoluta*.

18 month plan:

- No plan for the next 18 months.

Participants (Muhammad Zahid, Muhammad Salman & Alamzeb (Pakistan)

5 year plan:

- Assessment of irradiation doses for sterility of male fruit worm and subsequent mating compatibility with wild females in greenhouse.
- Evaluation of botanicals against larvae of fruit worm, *H. armigera* in greenhouse.
- Ecological studies of fruit worm, *H. armigera* through pheromone baited traps in tomato & okra crops in green house.

18 month plan:

- No plan for the next 18 months.

2.5. Sex Separation

It is worth mentioning that in Lepidoptera both sexes are released, unlike in many Diptera. Also, the females are more sensitive to radiation where the substerlizing dose for male moths may fully sterilize the females. Studying sexing systems in the lepidopteran insect pests is very important in order to lower programme costs.

Participants: Waheed Sayed, Alexandra Elhelaly, Gamal Hassan and Farha Hosny (EGYPT)

5 year plan:

- Traditional genetic methods conducted on *S. littoralis* and *T. absoluta* to develop a genetic sexing system
- To explore selectable markers using chemical mutagens
- To develop a translocation by low doses of gamma irradiation

18 month plan:

- No plan for the next 18 months.

3. TECHNICAL CONTRACTS

No decision was taken on the application of the technical contracts under the CRP at this stage. They will be reserved for use later as necessary.

4. WORKSHOPS

The two working groups are very separate, the main unifying factor being the greenhouse environment, including the influence of the external environment on infestation of the greenhouse and degree of isolation of the greenhouse from the external environment.

Possible common themes for the two working groups include:

- Rearing and QC methodologies,
- Colony management, genetic refreshing,
- Release systems in greenhouses,
- Irradiadiation protocols, dosimetry etc.

One or two short workshops around these themes will be developed to be run in conjunction with the second RCM.

5. SECOND RCM LOCATION

Gustavo Taret proposed holding the second RCM at SENASA/ISCAMEN, Mendoza, Argentina in the 1st quarter of 2019. Gustavo will suggest possible dates for ISCAMEN and then send a Doodle poll to the participants.

LOGICAL FRAMEWORK

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
Overall Objective: To advance development and implementation of SIT and inherited sterility for integration with other biocontrol for greenhouse and other confined arthropod pests	N/A	N/A	Non-SIT biocontrol is not sufficiently controlling the targeted pests in confined cropping systems
Specific Objective: To adapt inherited sterility or SIT for Tuta, Spodoptera and Helicoverpa species for confined cropping systems To develop SIT for *Drosophila suzukii* To develop inherited sterility or SIT for *Tuta absoluta*, Spodoptera littoralis and Helicoverpa armigera	Techniques advanced Network established	Reports and publications of techniques Number, expertise and geographic distribution of applicants	Regulatory requirements permit the use of inherited sterility Suitable participants apply to join the CRP with a broad range of expertise User community is engaged Radiation services and insects colonies are available
Outcomes: 1. SIT and inherited sterility techniques for the targeted pest species ready for implementation in confined cropping systems			R&D has resulted in a functional SIT package for some of the targeted species

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
2. SIT and inherited sterility techniques for the targeted pest species adopted in confined cropping systems	Crop losses	National statistics	Growers are willing to adopt the developed technology Growers acceptance of limited crop damage from F1 sterility No other sustainable control method will become available
Outputs:			
Survey on factors inhibiting the adoption of inherited sterility for Tuta, Spodoptera and Helicoverpa group	Survey conducted	RCM report	Industry engagement
Feasibility study on inherited sterility and SIT for Tuta, Spodoptera and Helicoverpa group in confined cropping systems	Research conducted	Research reports	Viable opportunities are identified New techniques are appropriate
Radiation biology for *D. suzukii*	Protocols	RCM report	Techniques developed
Sexing system for *D. suzukii*	Protocols	RCM report	
Mass rearing for *D. suzukii*	Manuscripts drafted	Manuscripts submitted	Manuscripts accepted
Feasibility study for *D. suzukii* in confined cropping systems	New facts and refined understanding	Papers published, contract reports, CRP review	End users engaged
Radiation biology *T. absoluta*, *S. littoralis* and *H. armigera*	Protocols	RCM report	Techniques developed
Sexing system for *T. absoluta* and *S. littoralis*	Test conducted	Test reports	End users engaged

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
Mass rearing for *T. absoluta* , *S. littoralis* and *H. armigera* Feasibility study for *T. absoluta* , *S. littoralis* and *H. armigera* in confined cropping systems	Manuscripts drafted New facts and refined understanding Recommendations for future work	Manuscripts submitted Papers published, contract reports, CRP review RCM report	Manuscripts accepted End users engaged Validation will not be completed within the CRP period New opportunities identified as a result of the CRP Project is still relevant at the end of the CRP
Activities: 1. Submit CRP proposal. 2. Announce project to MS and amongst established entomologists, biocontrol and pest control specialists and commercial glasshouse growers 3. Organize first RCM to plan, coordinate and review research activities 4. Carry out R&D. 5. Second RCM to analyse data and draft technical protocols as required			Project is approved

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
6. Hold workshop on "Insect mass rearing for pests of confined cropping systems (*D. suzukii*) and irradiation protocols", in conjunction with second RCM. 7. Continue R&D. 8. Review the CRP after its third year. 9. Convene third RCM to evaluate and standardize protocols. 10. Hold workshop on "Insect mass rearing for pests of confined cropping systems (Lepidoptera) and irradiation protocols", in conjunction with third RCM. 11. Continue R&D. 12. Hold final RCM to review data and reach consensus. 13. Evaluate the CRP and submit evaluation report. 14. Summarize and publish advances of CRP in a series of joint publications (journal special issue).			

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ANNEX 1 (LIST OF PARTICIPANTS)

1st RCM on Integration of the Sterile Insect Technique with Biocontrol for Greenhouse Insect Pest Management (D4 30 03)

3-7 July 2017, Vienna, Austria

1. ARGENTINA

Mr Gustavo Abelardo Ariel TARET

Boulogne Sur Mer 3050 1º piso
Ciudad, MENDOZA, CP5500
ARGENTINA

Email: gustavotaret@yahoo.com.ar

2. BRAZIL

Ms Alexandra KRÜGER
Departamento de Fitossanidade
Universidade Federal de Pelotas
PELOTAS RS BRAZIL
BRAZIL

Email: alexandra_Krüger@hotmail.com

3. CHILE

Ms Carolina Alejandra YANEZ BRICENO

Fundación para el Desarollo Frutícola
Los Coigües N° 651
SANTIAGO
CHILE

Email: cyanez@fdf.cl

4. CANADA

Ms Annabelle FIRLEJ

IRDA
335 rang des vingt cinq est
J3V0G7 St-Bruno-de-Montarville
QUEBEC
CANADA

Email: annabelle.firlej@irda.qc.ca

5. EGYPT

Mr Waheed Ahmed Abdelhamid SAYED

Nuclear Research Centre (NRC)
Atomic Energy Authority (AEA)
PO Box 9621
11787 CAIRO
EGYPT

Email: waheedel@hotmail.com

6. ITALY

Mr Massimo CRISTOFARO

Biotechnology and Biological
Control Agency BBCA onlus
Via Angelo Signorelli 105
00123 ROME
ITALY

Email: m.cristofaro55@gmail.com

7. ITALY

Ms Anna Rodolfa MALACRIDA

Dipartimento de Biologia e Biotechnologie
Universita degli Studi di Pavia
Via Ferrata 9
27100 PAVIA
ITALY

Email: annarodolfa.malacrida@unipv.it

8. INDIA

Mr Vayalam P. VENUGOPALAN

Nuclear Agriculture & Biotechnology Division
Bhabha Atomic Research Centre, Trombay
MUMBAI 400085
INDIA

Email: vpvenu@barc.gov.in

9. MEXICO

Mr Victor Manuel GUTIERREZ PALOMARES

Instituto de Sanidad y Calidad Agropecuria Mendoza
Av. Boulogne Sur Mer 3050
MENDOZA
MEXICO

Email: dgsv.cnrfito20@senasica.gob.mx

10. PAKISTAN

Mr Muhammad ZAHID

Nuclear Institute for Food and Agriculture (NIFA)
Pakistan Atomic Energy Commission (PAEC)
P.O. Box 446, G.T. Road
PESHAWAR 25000
PAKISTAN

Email: zahidnifa200028@yahoo.com

OBSERVERS

11. AUSTRIA

Ms Inessa KALANTAROW

Center for Health & Bioresources
Austrian Institute of Technology GmbH
TULLN
AUSTRIA

E-mail: Inessa.Kalantarow@ait.ac.at

12. CHINA

Mr Zeng-Rong ZHU
Institute of Insect Sciences
AgroSciences Complex C1045 Zijinggang Campus
Zhejiang University
HANGZHOU, ZHEJIANG, 310058
CHINA

Email: zrzhu@zju.edu.cn

13. FRANCE

Mr Allan DEBELLE

Center for Biology and Management of Populations (CBGP)
Institute for Agronomic Research INRA
MONTPELLIER
FRANCE

E-mail: allandebelle@gmail.com

ANNEX 2 (AGENDA)

FIRST FAO/IAEA RESEARCH COORDINATION MEETING ON

Integration of the SIT with Biocontrol for Greenhouse Insect Pest Management

3 – 7 July 2017, Vienna, Austria

Vienna International Centre (IAEA Headquarters), Building M - Room M6

AGENDA

Monday, 3 July, 2017

SESSION 1

08.00-09. 00	Registration at Gate 1 (by underground U1 station)
09.00-09.15	Andrew Parker and Carlos Caceres: Welcome and introduction
09.15-09.30	Administrative details
09.30-10.00	Nathalie Colinet: The IAEA's Research Contracts Activities
10.00-10.15	COFFEE

SESSION 2

10.15-10.35	Fabiana Sassu and Carlos Caceres: Progress on the development of SIT for SWF at IPCL.
10.35-11.00	Katerina Nikolouli, Laurence Mouton, Christian Stauffer and Kostas Bourtzis: Could insect genetics and symbiosis contribute to the development of SIT-based applications against *Drosophila suzukii*?
11.00-11.30	Alexandra Krüger: The use of SIT for *Drosophila suzukii* in Brazil

11.30-12.00 Carolina Yáñez: Combining inherited sterility and *Trichogramma* egg parasitoid to suppress *Tuta absoluta* population in tomato greenhouses

12.00-13.00 LUNCH

13.00-13.30 Annabelle Firlej: Research program on *Drosophila suzukii* control and sterile insect technique

13.30-14.00 Waheed Sayed: Increased Effectiveness of Sterile Insect Technique for Control of *Tuta absoluta* and *Spodoptera littoralis* in Greenhouses through F1 Sterility Combined with *Trichogramma* Parasitoid and/or Baculovirus

14:00- 14:30 Massimo Cristofaro, Silvia Arnone, Claudio Ioriatti, Gianfranco Anfora, Francesca Marini: Preliminary bioassays to evaluate the suitability of a SIT program to control *Drosophila suzukii* in greenhouse conditions in Italy

14.30-14.45 COFFEE

14.45-15.15 Anna Malacrida: Genetic and molecular approaches to identify traits of *Drosophila suzukii* reproductive biology which may impact the adoption of SIT in greenhouses

15.15-15.45 Ashok B. Hadapad, Ramesh S. Hire and V. P. Venugopalan: Integration of Sterile Insect Technique with biocontrol agents for the management of Tomato Leaf Miner, Tuta absoluta.

15.45-16.15 Victor Gutierrez Palomares: Integration of SIT with biocontrol for spotted wing (*Drosophila suzukii*) management in Greenhouse, in strawberry

16.15-17.30 General discussion

Tuesday, 4 July, 2017

SESSION 3

08:30- 09:00 Muhammad Zahid: Environment friendly management of fruit worm, *Helicoverpa armigera* (Hub.) through *Trichogramma chilonis* (Ishii) coupled with sterile insect technique (SIT) in tomato & okra crops in greenhouse and field conditions

09.00-09.30 Gustavo Taret: Development of Sterile Insect Technique for *Drosophila suzukii*

09.30-10.00 Zeng-Rong Zhu: Establishment of an RNAi-based RIDL (Release of Insects Carrying a Dominant Lethal) system in *Drosophila suzukii* to suppress its population growth

10.00-10.30 COFFEE

10.30-11.00 Allan Debelle: Male performance differences between artificial and natural conditions

11:00- 11:30 Pablo Montoya Gerardo, Emilio Hernández, Marysol Aceituno-Medina, Hugo Arredondo, Samuel Pineda: Effectiveness of the Sterile Insect Technique (SIT) to control *Drosophila suzukii* (Matsumura) under greenhouse conditions.

11.30-12.00 General Discussion

12.00-13.00 LUNCH

13.00-14.30 General Discussion of the Logical Framework

14.30-15.00 Selection of working group Chairs and Rapporteurs:
Drosophila suzukii. (M6)
Spodoptera/Helicoverpa and *Tuta absoluta* (A2411)

15.00-15.30 COFFEE

15.30-17.30 Working Group Discussions (M6/A2411)

Wednesday 5 July, 2017

SESSION 4

08.30-09.45 Working Group Discussions

09.45-10.15 COFFEE

10.15-12.00 Working Group Discussions

12.10-17.00 Visit to the Insect Pest Control Laboratory, Seibersdorf

17:00- 21:00 Group Dinner at Strandcafé (www.strandcafe-wien.at/en/)

Thursday 6 July, 2017

SESSION 5

09.00-09.40 Working Group Discussions

09.45-10.15 COFFEE

10.15-12.30 Working Group Discussions

12.30-13.30 LUNCH

13.30-15.30 Drafting Report

15.30-16.00 COFFEE

16.00-17.00 Drafting Report

Friday 7 July, 2017

SESSION 6

09.00-10.30 Reports of Working Groups

10.30-11.00 COFFEE

11.00-12.30 Drafting of RCM report

12.30-13.30 LUNCH

14.00-14.30 General Discussion

Closing

ANNEX 3 (WORKING GROUPS)

Spodoptera/Helicoverpa/Tuta	***Drosophila suzukii***
Carolina Yanez	Massimo Cristofaro
Waheed Sayed	Anna Malacrida
Muhammad Zahid	Zhu Zeng-Rong
V. P. Venugopalan	Fabiana Sassu
	Gustavo Taret
	Alexandra Krüger
	Victor Gutierrez
	Annabelle Firlej
	Aikaterini Nikolouli
	Allan Debelle

ANNEX 4 (ABSTRACTS OF PRESENTATIONS)

PRELIMINARY BIOASSAYS TO EVALUATE THE SUITABILITY OF A SIT PROGRAM TO CONTROL *DROSOPHILA SUZUKII* IN GREENHOUSE CONDITIONS IN ITALY

AUTHOR (S): *Massimo Cristofaro*

ORGANIZATION: *Biotechnology and Biological Control Agency BBCA onlus*

SHORT SUMMARY OF PAPER

Abstract:

Spotted-wing drosophila (*Drosophila suzukii*) is a vinegar fly endemic to South East Asia, became recently an invasive alien pest in several European Countries, including Italy. The fly is a pest of several soft summer fruit species and the infestation occurs at the ripening stage, preferred to the rotting fruit stages. Most damage caused by *D. suzukii* is due to larvae feeding in fruit flesh, even if the serrated ovipositor can produce a physical damage and can provide access to secondary pathogenic diseases.

Aim of this four-year project is to carry out the necessary biological and behavioral observations to better understand the mating patterns and physiological trails to develop a laboratory rearing suitable on SIT strategy. The behavioral observations to evaluate the effects of different irradiation doses on the fitness and the sterility on spotted-wing drosophila will be carried out in laboratory and quarantine green houses at the BBCA facilities in cooperation with ENEA. Laboratory bioassays and preliminary confined-field studies will be carried out at the facilities of Foundation Edmund Mach of S. Michele all'Adige (TN), to evaluate the quality of irradiated flies comparing their competitiveness with non-treated adults for validating the suitability for a small-scale SIT program in greenhouse conditions.

MALE PERFORMANCE DIFFERENCES BETWEEN ARTIFICIAL AND NATURAL CONDITIONS

AUTHOR (S): *Dr. Allan Debelle & Dr Simon Fellous*

ORGANIZATION: *Centre de Biologie pour la Gestion des Populations (CBGP; Montpellier, France)*

SHORT SUMMARY OF PAPER

Abstract:

The effectiveness of SIT programs is dependent on the performance of the sterile males released in the field. Thus, detailed knowledge about male and female sexual behaviour in realistic conditions is needed to identify the key male characteristics behind high mating success in the field. Such knowledge is yet largely lacking for many important pest species, and particularly *Drosophila suzukii*.

In this project, I will study both male-male and male-female interactions in *D. suzukii* in both artificial and semi-natural conditions. By using tools such as social network analysis, I will provide detailed information about male-male and male-female interactions within a group, and compare those interactions between artificial and semi-natural conditions, to isolate male characteristics associated with increased mating success in semi-natural conditions. This knowledge will have the potential to improve the field performance of *D. suzukii* strains, and thus the effectiveness of future *D. suzukii* SIT programs.

RESEARCH PROGRAM ON *DROSOPHILA SUZUKII* CONTROL AND STERILE INSECT TECHNIQUE

AUTHOR (S): *Annabelle Firlej, Ph.D.*

ORGANIZATION: *Institut de recherche et de développement en agroenvironnement (IRDA)*

SHORT SUMMARY OF PAPER

Abstract:

Integrated fruit production laboratory in IRDA (Quebec) is leading a research program on spotted wing drosophila (Drosophila suzukii) by studying different aspects of D. suzukii ecology and methods of control (population study, physical crop protection, control with natural/commercial enemies, and efficacy of repellents…). Since 2014, we have developed a project to evaluate potential of sterile insect technique for D. suzukii with different partners and achieved to establish the dose response for D. suzukii sterilisation and studied the competitiveness of male D. suzukii irradiated in laboratory. Following presentation of those results, we will detail specific objectives of research proposed to the coordinated research program (CRP) that focus on: 1- irradiated male competitiveness in semi field cages, 2-flight ability of males irradiated, 3-compatibility of sterile male issued from lab rearing compared to wild and 4-influence of temperature and humidity on emergence of pupae and mating capacity of sterile males. Anticipated outcomes of the project are the development of sterile insect techniques for D. suzukii ready for starting modelling population dynamics and testing over flooding ratio.

INTEGRATION OF SIT WITH BIOCONTROL FOR SPOTTED WING (*DROSOPHILA SUZUKII*) MANAGEMENT IN GREENHOUSE, IN STRAWBERRY.

AUTHOR (S): *Ing. Víctor Manuel Gutiérrez Palomares, Dr. Jose Abel López Buenfil, M.C. Claudio Chavarín Palacio, M.C. Hugo Cesar Arredondo Bernal and M.C. Fabian Gutiérrez Chacón.*

ORGANIZATION: *SENASICA*

SHORT SUMMARY OF PAPER

Abstract:

The spotted-wing vinegar fly (*Drosophila suzukii*) is a pest which has caused rapid and dramatic alteration in integrated pest management (IPM) programs in susceptible fruit crops, and this requires significant resources and efforts to minimize the invasion of this pest.

In Mexico the presence of *D. suzukii* has been detected in some places of the states of Michoacán, Colima, Jalisco, Baja California, Aguascalientes, Guanajuato, Estado de México and Querétaro; All these detections have been made in traps with attractants, established in diverse crops. This pest is regulated for the export of berries from Mexico to China, where postharvest treatments are not considered to mitigate the risk of the pest.

The aim of this research is to evaluate, in a preliminary way, the sterile insect technique as a tool for the management of *D. suzukii*, combined with biological control, through the effect of entomopathogenic agents on sterile insects when are used as vectors and the effect of different irradiation doses on *D. suzukii*, on strawberry crops. The above will be done following the methodology of Probit, in order to evaluate the possible production of F1 sterile specimens of *D. suzukii*, by irradiation with Cobalt 60 (0, 60, 70, 80 and 90 Gy); for the biological control, strains Pf 21, Pf 17, Pf 15 and *Metarhizium anisopliae* will be used.

In 2017 advances, there are the establishment and increase of *D. suzukii* colony; the morphometric characterization to verify the identification of the genus and species of the colony and will also be used as a comparative means to determine the morphometric difference between irradiated and post irradiated specimens. On the other hand, another number of specimens will be introduced to the molecular laboratory to observe differences of DNA in pre and post irradiation.

THE USE OF SIT FOR *DROSOPHILA SUZUKII* IN BRAZIL

AUTHOR (S): *Alexandra Peter Krüger*

ORGANIZATION: *Universidade Federal de Pelotas*

SHORT SUMMARY OF PAPER

Abstract:

Drosophila suzukii has invaded many countries, and became a pest of soft-skinned fruits worldwide. In Brazil, since its first record in Brazil, our team develops research focused in a wide range of parameters regarding ecology, biology and control of *D. suzukii*, highlighting the first records of parasitoids, hosts and alternatives to chemical control. In addition, to our knowledge, our team is the first and the only group in Brazil investigating the effects of irradiation on sterility induction of the Spotted Wing Drosophila. Under the research project, our team intend to assess the effects of remating in fertility recovery; and the effects of abiotic factors on sterile insects, measure the competitiveness of sterile insets, develop sampling methods to estimate catch rate in greenhouses; determine optimum ratio and frequency for sterile insects' release and investigate the integration of biological control and Sterile Insect Technique (SIT). By the end of the project we expect to provide information about the use of SIT for control of *D. suzukii* in greenhouses as well as the applicability of using this control method integrated to *Trichophia anastrephae.*

GENETIC AND MOLECULAR APPROACHES TO IDENTIFY TRAITS OF DROSOPHILA SUZUKII REPRODUCTIVE BIOLOGY WHICH MAY IMPACT THE ADOPTION OF SIT IN GREENHOUSES

AUTHOR (S): *Anna R. Malacrida*

ORGANIZATION: *Department of Biology and Biotechnology, University of Pavia, Pavia, Italy*

SHORT SUMMARY OF PAPER

Abstract:

The knowledge we have accumulated in fruit fly biology and the technologies we have developed in tephritid fruitflies will be applied to *Drosophila suzukii*. Our research interest will be addressed to clarify some aspects of *D. suzukii*i reproductive biology, which may create constrains in adopting SIT.

In particular, we will evaluate:

- the presence and extent of polyandry and polygyny in different strains and populations
- the sperm use by the female both in the laboratory and in a greenhouse context
- the impact of different larval diets on the production of volatile components emitted by males and females, which can impact mating interactions.

ENVIRONMENT FRIENDLY MANAGEMENT OF FRUIT WORM, HELICOVERPA ARMIGERA *(HUB.) THROUGH* TRICHOGRAMMA CHILONIS *(ISHII) COUPLED WITH STERILE INSECT TECHNIQUE (SIT) IN TOMATO & OKRA CROPS IN GREENHOUSE AND FIELD CONDITIONS.*

AUTHOR (S): *Muhammad Zahid, Pr. Scientist/ CSI-(CRP-21002/RO)*

ORGANIZATION: *Plant Protection Division, NIFA, Peshawar, Pakistan*

SHORT SUMMARY OF PAPER

Abstract:

Fruit worm, *Helicoverpa armigera* (Hub.) is the major insect pest of tomato and okra crops in Pakistan. All crops are under severe attack of insect pests which result in heavy losses to growers. Pakistan imports pesticides more than worth of US 500-800 millions for the control of insect pests of crops, fruits & vegetables annually. Most of our farmers are applying insecticides which have resulted in insect resistance, cost of protection, pollute environment/water, health hazards and parasitoids, predators are adversely affected. Egg parasitoid, *Trichogramma* Spp. is the most important bio-control agent in many agricultural systems worldwide for the control of different insect pests in egg stage. *Trichogramma* is reared on fictitious host of Angoumois moth, *Sitotroga cerealella* (Oliv.) in laboratory conditions and their periodic releases made against eggs of fruit worm in tomato & okra. Bio-control is an important IPM component which is successfully use with sterile insect technique (SIT), pheromones baited traps and botanical pesticides against fruit worms and borers. SIT is the release of large numbers of sterile male moths to mate with wild females, thereby reducing their reproductive potential and also resulting in suppression/ eradication of the same species in the target areas.

Different experiments will be performed to assess and standardize the effectiveness of bio-control agent, *Trichogramma* during (2017-18) i.e., (a). Improvement and establishment the insectary of bio-control agents, *Trichogramma chilonis* (Ishii) on mass scale at laboratory. (b). Effect of the age of *S. cerealella* eggs on oviposition preference of the female parasitoid, *T. chilonis*. (c). Parasitism of *S. cerealella* eggs as influenced by the age of the female parasitoid, *T. chilonis*. (d). Oviposition preference of the female parasitoid, *T. chilonis* on different colour cards of host eggs, *S. cerealella*. (e). Impact of gamma radiation on hatching of *S. cerealella* and also egg parasitization of *T. chilonis*. (f). Effect of chilling on egg parasitization of *T. chilonis* and hatching of *S. cerealella* on production capabilities of *Trichogramma chilonis* and *Sitotroga cerealella* at Bio-control laboratory of Nuclear Institute for Food and Agriculture (NIFA).

DEVELOPMENT OF STERILE INSECT TECHNIQUE FOR *DROSOPHILA SUZUKII* (MATSUMURA)

AUTHOR(S): *Gustavo Taret [1-2], Wilda Ramirez [1], Mariel Vanin[2] , Esteban Garavelli [1], Florencia Vazquez[1], Paola Fedyszak [1] and Ana María Van Cauvlaert [2]*

ORGANIZATION: [1] *Servicio Nacional Sanidad y Calidad Agroalimentaria (SENASA)*
[2] *Instituto de Sanidad y Calidad Agropecuaria de Mendoza, ISCAMEN, Argentina.*

SHORT SUMMARY OF PAPER

Abstract:

The positive detection of *Drosophila suzukii* (Matsumura), in Argentina has generated a series of actions for the monitoring of the pest at the same time to evaluate the possible actions for the control of the pest. Being that it is a species that affects the fruits practically at the moment of harvest, the action of chemical control is very limited. At the same time the high reproductive power added to its short cycle, it forces to explore tools of control that are friendly with the environment at the same time of effective. The effectiveness of TIE for the control of D. suzukii requires an in-depth analysis to determine the certain possibility of its use on a massive scale, as well as to determine the bio-ecological environment where the sterile insect would take action. At the same time, the observation of the increasing damage in the farms with hosts of this pest accompanied by the request of aid of the producers on control technologies, makes necessary to analyze in depth the control of *Drosophila suzukii*.

The general objective of this project is to determine the feasibility of the use of SIT in the control of *Drosophila suzukii*, to be used in Argentina The present project addresses the need of the productive sector to develop technologies for the integrated management of Drosophila suzukii with the minimum environmental impact. The Sterile Insect Technique could be an excellent control tool to be developed under IPM concept. There are four phases in this project. The first is joined to the development of rearing process, quality control, irradiation, packaging and release system. The following steps are the application of the SIT and evaluation of the technique in farms with crops in greenhouse and open field. Argentina has blueberries, red berries, cherries, grapes, figs, with different farming systems (greenhouses and open field under intensive system-cost benefit ratio will be evaluated). As a result of this project, it is expected to develop and validate the use of SIT integrated with other control systems for D. suzukii in Argentina. Otherwise provide a technological package to growers with crops in greenhouse that they can validate it on their farms, with assistance of SENASA regionally.

INTEGRATION OF STERILE INSECT TECHNIQUE WITH BIOCONTROL AGENTS FOR THE MANAGEMENT OF TOMATO LEAF MINER, *TUTA ABSOLUTA*

AUTHOR (S): *Ashok B. Hadapad, Ramesh S. Hire and V. P. Venugopalan*

ORGANIZATION: *Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre (BARC), Trombay, Mumbai 400085 India*

SHORT SUMMARY OF PAPER

Abstract:

Tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a serious pest on tomato cultivation in South America, Mediterranean basin and Europe. This insect pest is very invasive and causes devastation of tomato crop in open and protected conditions. In India, *Tuta* has been observed for the first time in Maharashtra state in the year 2014. Later, infestation of *Tuta* has been reported from different states, where it caused 90-100% tomato fruit damage. Its aggressive and multivoltine nature, short generation time and high fecundity are some of the important factors that confer key pest status in new localities. Currently, selected insecticides, pheromone traps, biorational insecticides and egg parasitoids are being tested for suppression of *Tuta* under outdoor and protected conditions. Sterile Insect Technique (SIT) is one of the important control methods presently used worldwide for fruit fly and other lepidopteran insects. BARC has developed sterile insect technique for the control of agricultural crop pests like red palm weevil and potato tuber moth and has demonstrated its effectiveness in field. In order to develop SIT against tomato leaf miner, it is essential to fill the knowledge gap in terms of its actual distribution, mass rearing procedure, optimisation of sub-sterility dose and compatibility with other control strategies. In this CRP, it is proposed (1) to undertake studies to understand the distribution and genetic diversity of tomato leaf miner prevalent in India, (2) to standardise a mass rearing protocol to ensure the supply of insects, (3) to optimise sterility dose and (4) to carry out field evaluation for validation with and without other control strategies.

INCREASED EFFECTIVENESS OF STERILE INSECT TECHNIQUE FOR CONTROL OF *TUTA ABSOLUTA* AND *SPODOPTERA LITTORALIS* IN GREENHOUSES THROUGH F_1 STERILITY COMBINED WITH TRICHOGRAMMA PARASITOID AND/OR BACULOVIRUS

AUTHOR (S): *Sayed, A. A. Waheed*

ORGANIZATION: *Biological Application Department, Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt*

SHORT SUMMARY OF PAPER

Abstract:

The tomato leaf miner, *Tuta absoluta* (Meyrick) have been listed on the invasive pest in several countries in many regions, causing severe loss for Solanaceous crops especially tomato production either in open field or in greenhouses. In addition, the cotton leaf worm, Spodoptera littoralis (Boisd.) is one of the main pest around the world, it has a wide range of hosts, feeding on 100 species are known in Egypt. Several bio-control agents are used to control these pests such as *Trichogramma* parasitoid and Baculovirus, however supplementary methods are still needed to achieve an adequate level of control. Sterile Insect Technique is considered as an environmentally compatible for insect pest management, inherited sterility (IS) offers significant advantages over classical sterile released method for lepidopteran pests. The effect of various radiation dose levels on the resulting sterility in the F1 progeny and the mating competitiveness will be studied on laboratory and field cages to assess the impact of IS on *T. absoluta*, in addition, the mating competitiveness of the sterile S. littoralis moths will be carried out in the field cages to determine the optimal dose of gamma irradiation. For successful SIT/IS application, development of *T. absoluta* diets using locally available ingredients as well as improvement the *S. littoralis* mass rearing are needed to take advantage of the economy of scale. Furthermore, the possibilities of sex separation of the two tested insect would reduce the rearing costs especially those that would eliminate females early in egg or larval stage, the genetic sexing of tested insect using classical genetic will be explored for development a genetic sexing strains could release only male to eliminate assortative mating of released moths. On the other hand, greenhouses experiments of using SIT/IS and *Trichogramma* for *T. absoluta* management will be conducted as well as the possibilities of isolate *T. absoluta* Baculovirus. In addition, the feasibility studies of using SIT/IS combined with *Trichogramma* and/or nucleopolyhedro virus (SpliNPV) are very important for successful integrated *S. littoralis* management in greenhouses

COMBINING INHERITED STERILITY AND *TRICHOGRAMMA* EGG PARASITOID TO SUPPRESS *TUTA ABSOLUTA* POPULATION IN TOMATO GREENHOUSES.

AUTHOR (S): *Carolina Yáñez*

ORGANIZATION: *Fundación para el Desarollo Frutícola, Los Coigües N° 651, Santiago, Chile*

SHORT SUMMARY OF PAPER

Abstract:

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is one of the most destructive insect pest to tomato plants and fruits worldwide. Currently, the insect is controlled by the application of highly hazardous and broad-spectrum insecticides. The effects of ionizing irradiation (Inherited Sterility technique) on *T. absoluta* as a potential control tactic had been previously studied by Cagnotti et al. (2012). A dose of 200 Gy was proposed as an optimum dose for the species for use in an area-wide integrated pest management. In recent research, Cagnotti et al. (2016) found, that a certain level of suppression of a *T. absoluta* wild population was achieved through the release of irradiated insects (males and females); and it was technically feasible to combine the use of an egg predator, (Hemiptera: Miridae), with inherited sterility to control *T. absoluta*.

Biological control against *T. absoluta*, by releasing *Trichogramma* parasitoids, is considered to be promising as a management tool for this pest. In South America, *T. absoluta* is parasitized by *Trichogramma pretiosum* Riley and *Trichogramma achaeae* Nagaraja and Nagarkatti under laboratory and greenhouse conditions. Moreover, a synergistic benefit has been demonstrated to occur when sterile eggs laid by irradiated female moths have been used to enhance the numerical response of egg parasitoids.

To the best of our knowledge, no study has been conducted to examine neither the acceptability/suitability of *T. absoluta* eggs from irradiated parents to be parasitized by *T. achaeae*, nor the effects of combining the Inherited Sterility technique and the release of *Trichogramma* (*T. achaeae*) to suppress *T. absoluta* population in tomato greenhouses. In contrast, these parameters have been studied for *T. nerudai* and *T. pretiosum*. Therefore, the main objectives of the proposed research are:

- Studying the parasitization of *T. absoluta* eggs from 200 Gy-irradiated parents (pupae) by *T. achaeae* with the choice and no-choice tests conducted under laboratory conditions.
- Evaluating the integration of Inherited Sterility with *T. pretiosum*, *T. nerudai* and *T. achaeae* to control *T. absulota* populations under tomato greenhouse environments.