

WORKING MATERIAL

Integration of SIT with Biocontrol for Greenhouse Insect Pest Management

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

Hotel Crillón, Mendoza, Argentina

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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 must 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; Merx-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 absoluta* group**

Tuta absoluta (tomato leaf miner) (Lepidoptera: Gelechiidae) is an emerging pest 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).

The objective of developing an SIT program for *T. absoluta* 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 this Solanaceous pest in the event it invades 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

SECOND RESEARCH CO-ORDINATION MEETING (RCM)

Fifteen scientists and several observers from 12 countries attended this second RCM, held in Mendoza, Argentina from 4-8 March 2019. 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 one and a half days of the meeting, RCM participants presented progress of their research relevant to the CRP, as well as their research plans for the next 18 months of the CRP.

During the last three days of the meeting, general discussions were held to define and review the thematic areas of the CRP (Table 1) and to review the general and specific R&D objectives to be addressed during the 5 years of the CRP and the CRP Logical Framework was reviewed, 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 next 18 months of the CRP.

Abstracts of the presentations are presented in Annex 4 and presentations were made available to all participants at the end of the RCM.

Table 1. Thematic areas in relation to pest species being addressed by participants of the CRP.

Species	Greenhouse trials	Mass rearing	Radiation effects	Integration with biocontrol	Biology	Standardized protocols
<i>Drosophila suzukii</i>	Gustavo Target Annabelle Firlej	Aikaterini Nikolouli Massimo Cristofaro Gustavo Taret Fabianna Sassu Victor Gutierrez	Massimo Cristofaro Anna Malacrida Fabiana Sassu Gustavo Taret Alexandra Krüger Victor Gutierrez Annabelle Firlej Aikaterini Nikolouli	Alexandra Krüger Massimo Cristofaro Victor Gutierrez Gustavo Taret Lloyd Stringer	Anna Malacrida Allan Debelle Massimo Cristofaro Gustavo Taret Alexandra Krüger	Massimo Cristofaro Anna Malacrida Fabiana Sassu Gustavo Taret Alexandra Krüger Victor Gutierrez Annabelle Firlej Aikaterini Nikolouli
<i>Tuta absoluta</i>	Carolina Yanez Waheed Sayed Silvia Lopez Cynthia Cagnotti	Carolina Yanez Waheed Sayed V.P. Venugopalan	Carolina Yanez Waheed Sayed V. P. Venugopalan Silvia Lopez Cynthia Cagnotti	Carolina Yanez Waheed Sayed V. P. Venugopalan Silvia Lopez Cynthia Cagnotti	Silvia Lopez Cynthia Cagnotti	
<i>Spodoptera littoralis</i>	Waheed Sayed	Waheed Sayed	Waheed Sayed	Waheed Sayed		
<i>Heliocoverpa armigera</i>	Muhammad Zahid Shiva Osouli	Muhammad Zahid Shiva Osouli	Muhammad Zahid Shiva Osouli	Muhammad Zahid Shiva Osouli		

1. STERILE INSECT TECHNIQUE FOR *D. SUZUKII*

***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 pheromone 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 O₂ (i.e. normoxia/hypoxia) is under evaluation (Lanouette et al. 2017; Sassu et al. submitted, Krüger et al. 2018). First experiments show that the dose-response for female pupal sterility

lies between 30-50 Gy, depending on atmospheric conditions (Lanouette et al. 2017; Sassu et al. submitted). 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 radiation 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. sukukii*. 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. sukukii*

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, Cuthbertson 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 the Insect Pest Control Laboratory (IPCL), Allan Debelle and Gustavo Taret)

Results from the previous 18 months

- Identification and validation of informative markers for assessing the presence of polyandry in the wild has been done. These markers have been validated as informative for genotyping of the progeny and for deriving information on the sperm use (in collaboration with Edmund Mach Foundation)
- Characterisation of strains in terms of genetic background has been completed (in collaboration with Edmund Mach Foundation)
- Assessment of the presence of polyandry in a wild North Italian population sampled in the summer season, i.e. at high population density
- Preliminary evidence has been given that the remated female used the sperm from the different mates (in collaboration with Edmund Mach Foundation)

18 month plan

- Characterisation of strains in terms of genetic background (in collaboration with Aikaterini Nikolouli and Konstantinos Bourtzis (Vienna))
- Evaluation of the polyandry level in different seasons (in collaboration with Annabelle (Canada), Gustavo (Argentina), Alexandra (Brazil), Emilio (Mexico) and Simone (Edmund Mach Foundation)).
- Evaluation of the polyandry level and sperm use in different strains, populations from different geographical origins (in collaboration with Annabelle (Canada), Gustavo (Argentina), Alexandra (Brazil), Emilio (Mexico)).
- Evaluation of sperm viability in spermathecae in relation to storage time
- Evaluate the impact of different larval diets on the production of male and female sexual chemical signals (in collaboration with IPCL)
- Identification of sex-linked markers

Participants: Allan Debelle and Simon Fellous (France)

5 year plan:

- **Field test:** pilot releases of sterile males in different types of strawberry production units in France
- **Fly-microbe interactions:** validate relevant microorganisms (yeast & bacteria) for tailored fly production, behavioural manipulation, monitoring and mass trapping, and their implementation in SIT programmes
- **Monitoring/trapping:** developing tailored trapping systems based on sex, mating status and microbiota

Results from previous 18 months:

- Visual and olfactory male traits potentially targeted by *D. suzukii* female mate choice have been studied (wing dark spot, wing interference patterns, cuticular hydrocarbons).
- Data have been collected on >1200 individual males (Montpellier population)
- Preliminary analyses show a limited role of olfactory signals at best, but a significant importance of the dark spot characteristics

18 month plan:

- **Data analysis:** Complete the analyses of the male mating traits in relation to mating success
- **Male-male competition:** identify the traits involved in male-male competition
- **Heritability and plasticity of male traits:** assess the potential for transferability of these traits to a rearing colony
- **Lab/field relative importance of male traits:** compare the effects of these traits between artificial and natural conditions
- **Male mating characteristics:** determine mating capacity, female remating period, last male sperm precedence and polyandry (in collaboration with Anna, Simone, Emilio)
- **Male competitiveness:** conduct competitiveness trials with sterile males in semi-natural conditions (Annabelle, Gustavo, Lloyd)

Participants: Massimo Cristofaro and Simone Puppato (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.
- Define basic biological parameters related to the female mating:remating interval, refractory period (in collaboration with Edmund Mach Foundation)

Results from the previous 18 months:

- Baseline laboratory experiments for assessing sperm use both in a wild and laboratory strain in *Drosophila suzukii*.
- Evaluation of polyandry in a wild-caught *Drosophila suzukii* (in collaboration with Anna Malacrida).

18 month plan:

- Evaluation of the polyandry level and paternity skew at different population densities in different seasons (in collaboration with Anna Malacrida and others as previously noted)
- Evaluation of sperm viability in spermathecae and seminal receptacle in relation to storage time (in collaboration with Anna Malacrida)
- Define basic biological parameters related to the female mating:remating interval, refractory period (in collaboration with Anna Malacrida, and others as previously noted)

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.

Results from the previous 18 months:

- A GIS system was developed and it is being applied at this time. A monitoring system with 103 *D. suzukii* traps were deployed and georeferenced. These traps are included in the official exploring network shared between ISCAMEN and SENASA.

18 month plan:

- Sampling protocols will be applied and introduced in the designed GIS system

1.3. *Drosophila suzukii* radiation biology

Participants: Massimo Cristofaro and Simone Puppato (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 collaboration with IPCL)

Results

- Evaluation of impact of irradiation on copula duration, fecundity, fertility in crosses between:
 - wild fertile females and males
 - wild fertile females and sterile males

18 month plan:

- Competitiveness tests with wild and sterile males at different ratios (collaborate with Gustavo, IPCL, Lloyd, Allan, Emilio, Victor, Alexandra).

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).

Results from the previous 18 months

- A protocol for assessing the impact of irradiation on sperm use, mating and remating frequency was developed (in collaboration with Edmund Mach Foundation).

18 month plan

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

Participants: Fabiana Sassu and Carlos Caceres (Vienna/IPCL)

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.

Results from the previous 18 months:

- We established a dose-response curve for male and female *D. suzukii* irradiated with gamma radiation under normoxia and hypoxia atmospheric conditions;
- We tested competitiveness of flies irradiated under normoxia and hypoxia conditions in the laboratory at one dose (220 Gy);
- We evaluated some of the quality control parameters of irradiated males under normoxia and hypoxia atmosphere conditions.

18 month plan:

- Continue testing competitiveness of flies irradiated under normoxia and hypoxia conditions at the respective sterilising doses.
- Assess fitness pre- post- shipment (with Simone FEM)

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).

Results from the previous 18 months:

- Pre and post irradiation quality control procedures were developed for: Weight of pupae, Emergence and Flight ability, Longevity under stress and biological dosimetry. Preliminary records should be confirmed.
- Competitiveness Fried Test, were evaluated using sterile and fertile flies from the lab colony. The dose evaluated on all treatments was 70 Gy. Competitiveness tests were evaluated with small plexiglass cages and field cages under controlled conditions.

18 month plan:

- Continue with pre and post irradiation quality control parameters because it should be confirmed using 140 – 170 - 200 Gy.
- Continue with competitiveness Fried Test
 - Source of flies
 - Sterile flies from the lab colony
 - Fertile flies “wild flies” kept under natural condition (fruit).
 - Dose to be evaluated: 140 – 170 - 200 Gy.
 - Environment conditions
 - Small plexiglass cages (lab conditions)
 - Field cages
 - Lab conditions
 - Field conditions (to be performed at the beginning of spring time)
 - Greenhouse (strawberries)
 - Natural conditions (to be performed at the beginning of spring time) in collaboration with Simon and Allan

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

5 year plan:

- Evaluation of the effects of sterilisation on female remating.
- Evaluation of the effects of abiotic factors (temperature and humidity) on irradiated *D. suzukii* adults.

Results from the previous 18 months:

- We evaluated the effects of sterilisation of both male and female insects on female remating. We found an overall low remating rate by *D. suzukii* females. Male sterility did not influence mating and remating likelihood; however, copula duration of sterile males was shorter compared to fertile males. On the other hand, sterile females were less likely to mate. The results from this research were presented in XXVII Congresso Brasileiro de Entomologia (oral presentations), and they were also published in Journal of Applied Entomology in 2019.
- We evaluated the effects of temperature and humidity on longevity and mating of irradiated *D. suzukii* adults. We found no difference between the longevity of irradiated and non-irradiated insects. However, insects are strongly affected by abiotic conditions, mainly high temperatures and low humidities.

18 month plan:

- completed

Participants: Victor Gutierrez (Mexico)

5 year plan:

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

Results from the previous 18 months:

- Different doses of gamma radiation (0, 60,70,80,90,180 and 200 Gy) were evaluated on 5 day old pupae of *D. sukuzii* for the quality parameters (Emergence, sexual ratio, emergence time, longevity time, fertility recovery and external morphometry). No significances differences were observed between treatments and control in parameters of (Emergence, sexual ratio, emergence time, longevity time). For fertility, significant differences were observed between the treatments and controls on female fertility recovery when a fertile female mated with an irradiated male and re-mated with a fertile male. And for external morphometry there was not clear tendency to relate the increase or decrease in the external size of the insect with the absorbed dose of radiation.

18 month plan:

- Evaluation of different densities of 200 Gy irradiated males for population control in confined conditions and greenhouse planted with strawberries (collaborate with Gustavo and Allan/Simon, Alexandra).
- Start the development of a packing, transporting and release system for sterile *D. sukuzii* (collaborate with Emilio and Fabiana and Simone)

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).

Results from the previous 18 months:

- We evaluated the competitiveness of sterile males at different radiation doses in laboratory and field cages; doses tested were 80-90-100-120 grays. In both experiments, among dose treatments, we compared combinations when 10 females were offered 20 sterile males, 20 fertile males or 10 sterile + 10 fertile males. In laboratory trials, we recorded egg hatching for 5 days and for field cages, we recorded the number of fruits infested and progeny produced. Results in the laboratory showed that sterilized males from 80 to 100 grays are mostly as competitive as fertile males, but sterilized males at 120 grays showed decreased competitiveness. In the presence of only irradiated males at 120 grays, sterility of eggs reached 98.1%. Results in field cages showed no reduction of fruit infested in competition treatments no matter the dose used even if progeny was reduced by 50% with 80 and 120 grays. In the presence of only irradiated males at 120 grays, percentage of infested fruits was 1.7%.
- We evaluated the effect of temperature (10, 15, 25, 30 and 35°C) on the emergence, survival and malformation of irradiated male at 80, 90, 100 and 120 grays. Results are partially available, and we observed that emergence was reduced to 68% for 10C degrees and to 7.6% for 35C degrees.
- We evaluated the timing of emergence of drosophila and flight capacity of sterile males. When pupae were irradiated at 80 and 120 grays, adults emerged mainly the morning following 48h after pupal irradiation. Flight capacity experiment results were not positive due to a problem in the set-up of the experiment.

18 month plan:

- Assess mating compatibility between wild and laboratory strains, as well as between strains of different geographical origins (in collaboration with Gustavo, Allan, Anna/Simone).
- Test the effects of different abiotic conditions on irradiated pupal survival and irradiated male mating success (In collaboration with Allan Debelle and Alexandra Krüger).
- Determine the effect of radiation doses on flight capacity and dispersal (in collaboration with Massimo Cristofaro).

Participants: Aikaterini Nikolouli and Konstantinos Bourtzis (Vienna)

5 year plan:

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

Results from the previous 18 months:

- Fitness and cytoplasmic incompatibility (CI) experiments was performed for all *Wolbachia* infected and uninfected lines of *D. sukii*.
- Effect of male age on CI was investigated.

- Competitiveness experiments were performed in population cages. Different ratios of males infected by wHa and wTei were tested, but results were inconclusive.

18 month plan:

- Repeat competitiveness experiments in small cages in the laboratory. Different ratios of males infected by wHa and wTei will be tested.

1.4. Mass rearing for *D. sukukii*

Participants: Pablo Montoya, Rubén Hernández, Marysol Aceituno-Medina, Emilio Hernández (México), in collaboration with Víctor Manuel Gutiérrez Palomares (México) and Gustavo Taret (Argentina).

5 year plan:

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

Results from the previous 18 months:

- The mass rearing colony was established in the Moscafrut Facility.
- Three larval diets were developed: Coconut fiber + Torula yeast, Coconut fiber + brewer's yeast, and Corncob fractions + brewer's yeast. The average transformation from eggs to adult was 25%.
- A cage for adults was developed using the cages for the longevity test of sterile *Anastrepha* spp. males.
- The feed conversion index was included as mass rearing parameter to evaluate the economic feasibility of the mass rearing, which indicated a low conversion of 2% with a pupal weight of 1.8 mg, 80% of adult eclosion and a yield of 12 larvae per gram of diet.

18 month plan:

- Develop egg collection and pupal separation technique (collaborate/communicate with IPCL).
- Measure colonisation effects in laboratory rearing.
- Improve the cage for adult oviposition (collaborate/communicate with IPCL).
- Improve the pupal yield.
- Determine the effect of the temperature on the sexual proportion.

- Start to develop a system for packaging, transporting and releasing sterile *D. sukuzii* (pupae or adults) (collaborate/communicate with IPCL).

Participants: Aikaterini Nikolouli and Konstantinos Bourtzis (Vienna)

5 year plan:

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

Results from the previous 18 months:

- The wild population was collected by October 2017 and a colony setup.
- DNA was extracted from adults from the following generations: F0, F1, F2, F5 and F10 and sent for microsatellite analysis.
- Gut dissections were done for the following generations: F0-F10, F15 and F20.

18 month plan:

- Analyse data from microsatellite analysis
- Analyse data from gut dissections.

Participant: Massimo Cristofaro (Italy)

5 year plan:

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

Result for the previous 18 months

- No research conducted

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

Results from the previous 18 months

- To develop egg collection and pupal separation technique.
 - A preliminary egg collection system was developed. However, it should be adjusted.
 - A pupal separation technique using a centrifugal prototype machine is under evaluation with our larval diet components. Damage to the pupa should be evaluated and also to check with other larval diet components.
- Measure colonisation effects in laboratory rearing. To be developed on the next 18 months.

18 month plan:

- Develop egg collection and pupal separation technique (in collaboration as mentioned previously)
- Measure colonisation effects in laboratory rearing. (in collaboration as mentioned previously)

Participants: Fabiana Sassu and Carlos Caceres (Vienna/IPCL)

5 year plan:

- Develop an oviposition system for *D. suzukii*.
- Evaluation of different artificial diets on larval/adult fitness.

Results from the previous 18 months:

- We developed an oviposition system for *Drosophila suzukii* egg collection

18 month plan:

- Test the effect of probiotics in the diet on fitness of *D. suzukii* (in collaboration with Aikaterini)

Participants: Allan and Simon (France)

18 month plan:

- **Fly-microbe interactions:** identify *candidate* relevant microorganisms (yeast & bacteria) for tailored fly production, behavioural manipulation, monitoring and mass trapping.

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:

- Identification of the best density to release *Trichopria anastrephae* in greenhouses
- Develop the combined use of SIT and a pupal parasitoid.

Results from previous 18 months

- There were no plans for the previous 18 months.

18 month plan:

- Identification of the best density to release *Trichopria anastrephae* in greenhouses to manage *D. suzukii*.

Participants: Massimo Cristofaro and Simone Puppato (Italy)

5 year plan:

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

18 month plan:

- Assessment of combination SIT with biological control by means of native parasitoids

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.

Results from the previous 18 months:

- The strains of CHE-CNRCB 307 of *Isaria javanica*, CHE-CNRCB 224 of *Metarhizium anisoplae* and CNRCB 168 of *Beauveria bassiana* were grow in fungus media and the quality parameters are being carried out.

18 month plan:

- A bioassay with fungal strains. Concentration of 1×10^8 sprayed on *D. suzukii* adult bodies to measure lethal time.
- Run treatments with 4 concentrations of the fungus 1×10^6 - 1×10^9 sprayed on *D. suzukii* adult bodies to measure lethal concentration and lethal time.

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).

Results from the previous 18 months:

- There was no plan from the previous 18 months.

18 month plan:

- Bioassay under greenhouses under natural conditions next spring (2019), checking level of damage on the fruit when management tools are used; Seasonal time (approx. 3 months)
 - SIT
 - SIT+ BC (*Dicyphus sp*)
 - SIT + BC + mass trapping for SWD

Participants: Lloyd Stringer (New Zealand)

5 year plan:

- Identify the optimal monitoring strategy or strategies for populations of *D. suzukii*
- Relate population density to catch
- Develop the model for the timing and number of BCAs required per release
- Merge and optimise the current population growth/phenology/management models for *D. suzukii* to include the effect and use of control tools
- Test the combined model with a CRP collaborator or agency attempting management/eradication
- Develop the tool to be easily used by growers with varying levels of expertise and access to control tools

Results from previous 18 months:

- Developed sex and age-structured discrete time model to simulate the effects of the different tools on population growth
- Modelled the probable effects of two types of mass trapping and SIT on population growth rates for tools used separately and used together
- The sampling efficacy of SWD lure was estimated and the probability of detecting flies from populations of different density was estimated
- Commenced estimating the likely population size based on catch of a specific number of SWD in trap(s) when a grid efficacy is known.

18 month plan

- Finalise the modelling to estimate the population of SWD population related to catch in a grid of traps of known efficacy.
- Revise population growth model with updated information provided by CRP participants, such as effects of SIT and BCAs.
- Validate model(s) with participant(s) (Gustavo)
- Start to develop the tool that can be used by growers to estimate population density based on catch in trap(s) and suggest tool use for management goal.

Participants: Allan DeBelle and Simon Fellous (France)

18 month plan

- **Socio-technical aspects:** Elaborate with farmer associations the modalities of SIT deployment with respect to current agricultural practices
- **Stings:** What is the effect of sterile female SWD (both irradiated and wild-sterile-mated) stings on fruit degradation (collaborate with Gustavo, Annabelle, Emilio, Victor)

2. STERILE INSECT TECHNIQUE FOR LEPIDOPTERA

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.

The results for the objectives are as follows

- 1- The level of infestation has been studied and evaluated in the participating countries. The level of infestation of *Helicoverpa*, *Spodoptera* and *Tuta* is significant in all the participating countries.

- 2- Various insect cultures like *T. absoluta* in Argentina, Chile and India, *H. armigera* in Iran, *S. littoralis* in Egypt, and *Trichogramma* cultures in Pakistan were collected from fields and established in laboratories.
- 3- Larval diets were developed for *T. absoluta* in Chile, *H. armigera* in Iran and *S. littoralis* in Egypt using locally available media ingredients, like chickpea, cowpea and yeast powder.
- 4- Mass rearing protocols like artificial diet, oviposition parameters have been developed for Tuta, Helicoverpa and Spodoptera insects and the insect pest populations have been maintained upto 15 generations.
- 5- The mass rearing protocols developed by participating countries will be shared. Artificial diets developed for *T. absoluta* will be shared among participating countries for mass rearing of insects.

Participants: Carolina Yanez, David Castro, Susana Izquierdo, Viviana Pavez and George Saour (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 parameters of reared insect.

Results of the first 18 months

- *T. absoluta* was collected from the infested tomato fields and reared on tomato plants.
- Four artificial diets were developed and tested for Tuta rearing compared to tomato plants.
- One of the four diet is found to be supporting the complete life cycle of *T. absoluta* but recovery was only 11%.

18 month plan:

- Improve diet to increase artificial breeding and perform quality control.

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 parameters of reared insect.

Results of the first 18 months

- Six larval diets based on the modifications of the semi artificial diet developed by Shorey and Hale (1965) were tested for *S. littoralis* rearing.
- Out of the six diets, diet based on castor leaf powder was most suitable for *S. littoralis* rearing followed by diet based on white kidney bean and brewer's yeast.
- Castor leaves for rearing the culture are not available throughout the year.

18 month plan:

- Enhancement the rearing technique of *S. littoralis* on semi artificial diet
- Maintaining the colony of *T. absoluta* under laboratory conditions
- Developing the rearing methods of *T. absoluta* on semi 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 leafminer populations.
- Establishment of mass rearing protocols to meet the supply and demand for SIT.

Results of the first 18 months

- Infestation level of *T. absoluta* was surveyed across 13 locations from 10 different states of India.
- Very high infestation (60-80%) was observed in western and southern Indian states.
- Genetic diversity studies using *mtCOI* gene analysis revealed that the *T. absoluta* populations across India are genetically identical.
- Mass rearing protocol using tomato seedling have been optimized.

18 month plan:

- Continue to collect *Tuta absoluta* samples from Northern and Eastern parts of India using TLM lures.
- Assessment of genetic diversity of collected tomato leaf miner populations using additional molecular markers.
- Improvement of mass-rearing protocols for *Tuta absoluta*.

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

Results of the first 18 months

- Low temperature (cold) increased the shelf-life of *S. cerealella* and *T. chilonis* without maximum detrimental effect on the quality of egg-parasitoid and host eggs.
- Low storage temperature (4°C) is very effective for maximum parasitization. It also prolongs the incubation period of *S. cerealella* eggs which ensures year around availability of the host eggs.

18 month plan:

- Establishment and maintenance of *H. armigera* colony on natural and artificial diets

Participants (Shiva Osouli, Mehrdad Ahmadi (IRAN))

5 year plan:

- Establishment of laboratory cultures of *H. armigera*.
- Development of mass rearing protocols for *H. armigera*

Results of the first 12 months:

- The artificial diets and rearing system have been developed. Three different diets each containing a particular kind of meal (cowpea, pinto bean and chickpea) were used together or one after another it improves the quality of insects in mass rearing.

18 month plan:

- Mass rearing of *H. armigera* will be continued.

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 successfully managed 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 substerilizing 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 summarized as follow:

1. Determine the sterilizing doses of target insect pests
2. Determine the sub-sterilizing doses of target insect pests

3. Optimise the radiation dose for lepidopteran insect pests
4. Determine the dose-response curve for different lepidopteran insect pests

The results for the objectives are as follows

1. The sterilizing doses for *H. armigera* (350 Gy) and *T. absoluta* (150-200 Gy) have been studied
2. Sub-sterility dose for *H. armigera* has been optimized in Iran and sub-sterility dose for Tuta will be optimised during next 18 months
3. Radiation doses to induce complete as well as F1 sterility has been optimised for *H. armigera* and *T. absoluta*
4. Dose-response curve for *H. armigera*, *T. absoluta* and *S. littoralis* will be completed in next 18 months.

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

5 year plan:

- Determine the sterilizing dose for *T. absoluta*
- Determine the sub-sterilizing dose for *T. absoluta*

Results of the first 18 months

- Two doses of ionizing radiation, 150 and 200 Gy, were evaluated on pupae of *T. absoluta*.
- In both cases the sterility index was very low therefore fertility was high.
- The sterility index was calculated by estimating fecundity and fertility of the treatment with respect to control.

18 month plan:

- Evaluate the viability of the offspring (F1) from parents irradiated with 3 doses (150, 200, 250 Gy).

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

5 year plan:

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

Results of the first 18 months

- Male and female pupae of *S. littoralis* were exposed to 5 different doses (80, 100, 120, 140, 160 Gy) to study the sterility dose.

- Parameters like egg hatching, larval duration, pupal duration and percentage adult emergence were studied for the crosses made between unirradiated male and irradiated female and irradiated male and unirradiated female and compared with normal crosses.
- The results indicated that the percentages of pupation and adult emergence are drastically decreased with the increase in gamma irradiation dose.

18 month plan:

- Studying the dose Optimization for *S. littoralis*
- Studying the dose Optimization for *T. absoluta*.

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

5 year plan:

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

Results of the first 18 months:

- No experiments were planned.

18 month plan:

- Evaluation of the effects of gamma radiation on different pupal ages of *T. absoluta*.
- Fitness evaluation of partially sterile males of *T. absoluta*.

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

5 year plan:

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

Results of first 18 months:

- No experiments were initiated.

18 month plan:

Dose optimization for male sterility in *H. armigera*.

Participants: Shiva Osouli, Mehrdad Ahmadi (Iran)

5 year plan:

- To study the radiobiology and competitiveness ability of treated male (parental and F1 generation)

Results of the first 12 months:

- The radiation dose of 350 Gy was found to be effective to induce sterility in male and female *H. armigera* when used to irradiate 8-day old pupae.
- The dose of 200 Gy induced sterility in F1 male and female progeny of *H. armigera* when male parents irradiated as 8-day old pupae.
- The male moths emerging from pupae irradiated with sub-sterilizing and sterilizing doses were as competitive as untreated ones in terms of mating and the calculated competitiveness value of the irradiated males in parental and F1 generations was within the acceptable range.

18 months plan

- The objective completed. No further plan.

Participants: Verônica Yusef, Cynthia Cagnotti, Silvia López (Argentina)

5 years plan:

- To determine the most suitable dose of gamma radiation applied to *Tuta absoluta* adults
- To identify the irradiated adults through: 1) the sperm morphology and 2) the genetic polymorphism (RAPD-PCR).

Results of the first 7 months

- Work is recently initiated.

18 months plan:

- To determine the most suitable dose of gamma radiation applied to *Tuta absoluta* adults

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 percentage *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: Carolina Yanez, David Castro, Susana Izquierdo, Viviana Pavez and George Saour (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.

Results of the first 18 months

- Exploratory tests were carried out with *G. legneri* and *T. nerudai*, these tests will continue for the next 18 months.

18 month plan:

- *Goniozus legneri* and *Trichogramma* sp. (irradiated and non-irradiated) in choice and no choice test.

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* nucleopolyhedrovirus (Spli NPV).
- Attempt to isolate *T. absoluta* viruses.

Results of the first 18 months:

- Neonate *S. littoralis* larvae treated with 1×10^3 PIBs died in the 5th instar and led to highest yield of NP virus.
- The results obtained showed that the two doses 40 and 60 Gy are effective doses for irradiating *S. littoralis* male pupae to produce F1 larvae very sensitive to SpliNPV
- The dose of 125Gy in combination with 50 and 100 ppm Bt protein resulted effective control of *S. littoralis*.
- The combination study of SIT and *Chrysoperla carnea* results showed that the 2nd instar larvae of *C. carnea* prefer un-irradiated eggs of *S. littoralis* over those irradiated with gamma radiation (100, 130 and 160 Gy) and also the lower dose (100Gy) was preferred over the higher 130 and 160 Gy.

18 month plan:

- Studying the combination treatment of SIT and natural enemies against *T. absoluta*
- Studying the combination treatment of SIT and pathogens against *T. absoluta*

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).

Results of the first 18 months:

- No experiments were planned.

18 month plan:

- Studying the parasitization of *T. absoluta* eggs by *Trichogramma* sp. in choice and no-choice tests.
- Impact of gamma radiation on hatching of *Corcyra* and *Spodoptera* eggs and on egg parasitization by *Trichogramma* sp.

Participants: Muhammad Zahid, Muhammad Salman and Alamzeb (Pakistan)

5 year plan:

- Impact of gamma radiation on hatching of *S. cerealella* and 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 on egg parasitization by *T. chilonis*.
- Effect of chilling on hatching of *S. cerealella* and on egg parasitization by *T. chilonis*.

Results of the first 18 months:

- It was evident from the experimental results that the significant differences exist among different *Sitotroga* eggs ages. Maximum parasitism of *Sitotroga* eggs age (12 hrs.) was recorded i.e. 71.50 % followed by 24 h (65.50 %), 48 h (47.50 %) & minimum was recorded in 72 h (34.25%). The developmental period of *T. chilonis* inside host eggs remains almost the same i.e., 14.0 days. It is concluded that fresh eggs ages (12-24 h) of *Sitotroga cerealella* (Oliv.) are most suitable for maximum parasitism by *Trichogramma chilonis* (Ishii) for 24 h exposure time.
- The results indicated that *T. chilonis* parasitized significantly different at 5% level of probability. Maximum parasitization of *Trichogramma* on host eggs was recorded up to (73.50 %) during 12 hours of its age followed by 24 h (62.00 %), 48 h (43.50 %). Later on, the parasitization decreased upto 22.00% after 72 hours. The developmental period of *T. chilonis* inside the host eggs remains almost the same i.e. 14.0 days. From *Trichogramma* production point of view, it is important to use younger parasites (12-24 hours age) to achieve maximum parasitism of *Sitotroga* eggs.
- It was observed that the maximum parasitization of host eggs of *S. cerelella* was recorded on green color card (45%) followed by yellow (36%), red (31%), white (30%) blue (27%) and least parasitization was recorded on black color card.
- Low radiation doses increased the shelf-life of *S. cerealella* and *T. chilonis* without significant detrimental effect on the quality of egg-parasitoid and host eggs.
- Radiation dose (10-20 Gy) is very conducive for egg hatching, adult emergence and incubation period (8-9 days) of *Sitotroga cerealella* eggs to get short term storage (3-5 days).
- Our results clearly showed that the *T. chilonis* parasitizing potential of *Sitotroga* eggs was higher in control as compared to lower radiation doses (10-20 Gy) but due to

radiation effect, the parasitizing period (turned black) were prolonged on 8th day as compared to control (4th day). The findings of the study will be helpful during transportation and storage.

18 month plan:

- Establishment and maintenance of *Trichogramma chilonis* (Ishii) on host eggs of *Sitotroga cerealella* (Oliv.) under in vitro conditions.
- Effect of radiation on pupae of the egg parasitoid, *Trichogramma chilonis* (Ishii)
- Low temperature effect on pupae of egg parasitoid, *Trichogramma chilonis* (Ishii)

Participants: Silvia López, Silvia Lanzavecchia, Cynthia Cagnotti, Claudia Conte (Argentina)

5 years plan:

Integration of SIT with Biocontrol methods

- To study the development and the fecundity of *Tupiocoris cucurbitaceus* (Hemiptera: Miridae) female (predator) fed on *Tuta absoluta* eggs from parents with the male irradiated.
- To study the predation of *Tuta absoluta* eggs by *Tupiocoris cucurbitaceus* nymphs in choice and no choice test under laboratory conditions.
- To study the parasitism of *Tuta absoluta* larvae by *Goniozus legneri* (Hymenoptera: Bethylinidae) in choice and no choice test under laboratory conditions.

Results of the first 7 months:

- Work is recently initiated

18 months plan:

- To study the development and the fecundity of *Tupiocoris cucurbitaceus* (Hemiptera: Miridae) female (predator) fed on *Tuta absoluta* eggs from parents with the male irradiated
- To study the predation of *Tuta absoluta* eggs by *Tupiocoris cucurbitaceus* nymphs in choice and no choice test under laboratory conditions.

2.4. Greenhouse Experiments

Current knowledge

The four participants will carry out greenhouse studies to implement SIT or inherited 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: Carolina Yanez, David Castro, Susana Izquierdo, Viviana Pavez and George Saour (Chile and Syria)

5 year plan:

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

Results of the first 18 months:

- No experiments were planned

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*.

Results of the first 18 months:

- No experiments were planned

18 month plan:

- Integration of SIT with natural enemies to suppress the population of *S. littoralis* and *T. absoluta* in green house
- Studying the combination treatment of SIT and pathogens against *S. littoralis* and *T. absoluta* in green house

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*.

Results of the first 18 months:

- No experiments were planned.

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.

Results of the first 18 months

- No experiments were planned.

18 month plan:

- Effectiveness of egg parasitoid, *Trichogramma chilonis* (Ishii) against host eggs of *Sitotroga cerealella* (Oliv.) eggs in high tunnel and tomato fields.
- Ecological study of tomato fruit worm, *Helicoverpa armigera* (Hub.) through pheromone baited traps in high tunnel/ field tomato.

Participants (Shiva Osouli, Mehrdad Ahmadi (Iran))**5 year plan:**

- To study the effect of releasing sterile (parental and F1 generation) *H. armigera* on greenhouse crops.
- To determine the effect of integration of sterile (parental and F1 generation) *H. armigera* with *Trichogramma* sp. on greenhouse crops.

Results of the first 12 months

- No experiments were planned.

18 months plan:

- Study of sterile *H. armigera* males under greenhouse conditions.
- Effect of integration of sterile *H. armigera* males with *Trichogramma* sp. under greenhouse conditions.

Participants: Silvia López, Silvia Lanzavecchia, Cynthia Cagnotti, Claudia Conte (Argentina)

5 years plan:

- Population suppression by irradiated *Tuta absoluta* males and females and their progeny combined with the use of a mirid predator (*Tupiocoris cucurbitaceus*)

Results of the first 7 months

- Work is recently initiated

18 months plan:

- No plans 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 substerilizing 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

Results of the first 18 months:

- No experiments were planned.

18 month plan:

- To explore selectable markers using chemical mutagens
- To develop a translocation by low doses of gamma irradiation

2.6. Reproduction analyses for IS implementation

Current knowledge

Parthenogenesis is relatively rare in the order Lepidoptera, only a few cases have been reported until now. Caparros Megido et al. (2012) described laboratory evidence of males and

females being produced from unfertilized eggs by *T. absoluta*. In addition, Abbes & Chermiti (2014) found *T. absoluta* parthenogenetic populations from Tunisia.

Participants: Silvia López, Silvia Lanzavecchia, Cynthia Cagnotti, Claudia Conte (Argentina)

5 years plan:

- To evaluate parthenogenesis in *T. absoluta* populations from Argentina
- To detect *Wolbachia* in *T. absoluta* populations from Argentina
- To identify and to characterize *Wolbachia* strain/strains by using molecular tools
- To detect the presence of other reproductive symbionts (*Spiroplasma* sp., *Cardinium* sp., *Rickettsia* sp., *Arsenophonus* sp.).

Results of the first 7 months:

- No evidence of parthenogenesis was obtained in the assessed populations of *T. absoluta* from Argentina (La Plata population, Buenos Aires province and Bella Vista population, Corrientes province).
- We have detected the presence of *Wolbachia* in a *Tuta absoluta* population from La Plata (Buenos Aires, Argentina) using 16S rRNA marker following the protocol described by Heddi et al. (1999).

18 months plan:

- To evaluate the capacity to reproduce parthenogenetically of new populations of *T. absoluta* from Argentina.
- *Wolbachia* prevalence: the number of individuals analyzed for La Plata will be increased and individuals from the new populations will be analyzed.
- To continue identification and genetic characterization of *Wolbachia* strain/strains by sequencing – MLST (5 genes) and *wsp*.

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

5 year plan:

- Screening of *Tuta absoluta* populations for presence of endosymbionts associated with reproductive abnormalities in wild and laboratory populations

Results of the first 18 months:

- Total 75 samples of tomato leafminer, *T. absoluta* collected from nine different locations were screened for presence of *Wolbachia* of which 32 were found to be positive.
- Phylogenetic and MLST analysis revealed that the *Wolbachia* strain belong to supergroup B which is parasitic in nature.

18 month plan:

- Continue to screen wild and laboratory Tuta populations for Wolbachia infections.

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. Possible topics include:

- Sex-related markers RNA Seq
 - Service = lab, fee for service
- Genotyping
 - Service = lab, fee for service
- Compatability studies
 - Service = lab, running trials
- Tuta population comparisons between geographic regions-
 - service =material collection and analysis

4. WORKSHOPS

The second workshop will be held on Lepidoptera in conjunction with the third RCM.

5. THIRD RCM LOCATION

Ramesh Hire proposed holding the third RCM in Mumbai, India in the 3rd-4th quarter of 2020. We will select possible dates and then send a Doodle poll to the participants.

6. LOGICAL FRAMEWORK

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
<p>Overall Objective:</p> <p>To advance development and implementation of SIT and inherited sterility for integration with other biocontrol for greenhouse and other confined arthropod pests</p>	N/A	N/A	Non-SIT biocontrol is not sufficiently controlling the targeted pests in confined cropping systems
<p>Specific Objective:</p> <p>To adapt inherited sterility or SIT for <i>Tuta</i>, <i>Spodoptera</i> and <i>Helicoverpa</i> species for confined cropping systems</p> <p>To develop SIT for <i>Drosophila suzukii</i></p> <p>To develop inherited sterility or SIT for <i>Tuta absoluta</i>, <i>Spodoptera littoralis</i> and <i>Helicoverpa armigera</i></p>	<p>Techniques advanced</p> <p>Network established</p>	<p>Reports and publications of techniques</p> <p>Number, expertise and geographic distribution of applicants</p>	<p>Regulatory requirements permit the use of inherited sterility</p> <p>Suitable participants apply to join the CRP with a broad range of expertise</p> <p>User community is engaged</p> <p>Radiation services and insect colonies are available</p>
<p>Outcomes:</p> <p>1. SIT and inherited sterility techniques for the targeted pest species ready for implementation in confined cropping systems</p>			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
<p>Outputs:</p> <p>Survey on factors inhibiting the adoption of inherited sterility for Tuta, Spodoptera and Helicoverpa group</p> <p>Feasibility study on inherited sterility and SIT for Tuta, Spodoptera and Helicoverpa group in confined cropping systems</p> <p>Radiation biology for <i>D. suzukii</i></p> <p>Mass rearing for <i>D. suzukii</i></p> <p>Feasibility study for <i>D. suzukii</i> in confined cropping systems</p> <p>Radiation biology <i>T. absoluta</i>, <i>S. littoralis</i> and <i>H. armigera</i></p> <p>Sexing system for <i>T. absoluta</i> and <i>S. littoralis</i></p>	<p>Survey conducted</p> <p>Research conducted</p> <p>Protocols</p> <p>Manuscripts drafted</p> <p>New facts and refined understanding</p> <p>Protocols</p> <p>Test conducted</p>	<p>RCM report</p> <p>Research reports</p> <p>RCM report</p> <p>Manuscripts submitted</p> <p>Papers published, contract reports, CRP review</p> <p>RCM report</p> <p>Test reports</p>	<p>Industry engagement</p> <p>Viable opportunities are identified</p> <p>New techniques are appropriate</p> <p>Techniques developed</p> <p>Manuscripts accepted</p> <p>End users engaged</p> <p>Techniques developed</p> <p>End users engaged</p>

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
Mass rearing for <i>T. absoluta</i> , <i>S. littoralis</i> and <i>H. armigera</i>	Manuscripts drafted	Manuscripts submitted	Manuscripts accepted
Feasibility study for <i>T. absoluta</i> , <i>S. littoralis</i> and <i>H. armigera</i> in confined cropping systems	New facts and refined understanding Recommendations for future work	Papers published, contract reports, CRP review RCM report	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
Reproductive analysis for IS implementation	New facts and refined understanding	Papers published, contract reports, CRP review	Resources available
<p>Activities:</p> <ol style="list-style-type: none"> 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. 			Project is approved

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
<p>5. Second RCM to analyse data and draft technical protocols as required</p> <p>6. Hold workshop on "Insect mass rearing for pests of confined cropping systems (<i>D. suzukii</i>) and irradiation protocols", in conjunction with second RCM.</p> <p>7. Continue R&D.</p> <p>8. Review the CRP after its third year.</p> <p>9. Convene third RCM to evaluate and standardize protocols.</p> <p>10. Hold workshop on "Insect mass rearing for pests of confined cropping systems (Lepidoptera) and irradiation protocols", in conjunction with third RCM.</p> <p>11. Continue R&D.</p> <p>12. Hold final RCM to review data and reach consensus.</p> <p>13. Evaluate the CRP and submit evaluation report.</p>			

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
14. Summarize and publish advances of CRP in a series of joint publications (journal special issue).			

7. REFERENCES FROM CRP PARTICIPANTS

Journal

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Conference

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Krüger A.P., D.C.H. Schlesener, L.N. Martins, J. Wollmann, M. Depra and F.R.M. Garcia. Efeito da esterilidade de *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) sobre a recópula das fêmeas. XXVII Congresso Brasileiro de Entomologia e X Congresso Latino-Americano de Entomologia 2-6 September, Expogramado, Gramado/RS, Brazil.

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ANNEX 1 (LIST OF PARTICIPANTS)

2st RCM on Integration of the Sterile Insect Technique with Biocontrol for Greenhouse Insect Pest Management (D4 30 03)

4-8 March 2018, Mendoza, Argentina

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ANNEX 2 (AGENDA)

SECOND FAO/IAEA RESEARCH COORDINATION MEETING ON

Integration of the SIT with Biocontrol for Greenhouse Insect Pest Management

4 – 8 March 2019, Mendoza, Argentina

Hotel Crillón, Peru 1065, Mendoza

AGENDA

Monday, 4 March 2019

SESSION 1

08:30 - 09:00 Walk to Hotel Carillon

09:00 - 09:15 **Andrew Parker and Carlos Caceres:** Welcome and introduction

09:15 - 09:30 **OPENING** - [Government of Mendoza representative]

SESSION 2 D. SUZUKII PRESENTATIONS

09:30 - 09:55 **Gustavo Taret:** *Rearing and quality control of Drosophila suzukii*

09:55 - 10:20 **Fabiana Sassu:** *Development of mass rearing and quality control protocols for Drosophila suzukii for SIT application*

10:20 - 10:35 **COFFEE**

10:35 - 11:00 **Annabelle Firlej:** *TBA*

11:00 - 11:25 **Allan Debelle:** *Mating signals underlying male mating success in D. suzukii*

11:25 - 11:50 **Alexandra Kruger:** *Implications of sterility of Drosophila suzukii on mating and survival at different temperatures and humidities*

11:50 - 12:50 **LUNCH**

12:50 - 13:15 **Simone Puppato:** *Preliminary assessments towards the implementation of Sterile Insect Technique: baseline experiments for evaluating sperm utilization in Drosophila suzukii*

- 13:15 - 13:40 **Anna Malacrida:** *Markers for assessing the mating behaviour of Drosophila suzukii*
- 13:40 - 14:05 **Victor Manuel Gutierrez Palomares:** *SIT and biological control in confined conditions against D. suzukii in Mexico, preliminary advances*
- 14:05 - 14:30 **Emilio Hernandez Ortiz:** *Mass rearing, quality parameters and feed conversion in Drosophila suzukii (Diptera: Drosophilidae), and their economics aspects*
- 14:30 - 14:45 **COFFEE**
- 14:45 - 15:10 **Lloyd Stringer:** *Sampling for Drosophila suzukii*

SESSION 3 LEPIDOPTERA PRESENTATIONS

- 15:10 - 15:35 **Cynthia Cagnotti:** *Does parthenogenetic reproduction exist in populations of the leafminer Tuta absoluta (Lepidoptera: Gelechiidae) from Argentina?*
- 15:35 - 16:00 **Susana del Pilar Izquierdo Carreño:** *TBA*
- 16:00 - 16:25 **Waheed Ahmed Abdelhamid Sayed:** *Evaluation of simultaneous treatments of sterile insect technique and biological control agents for Spodoptera littoralis management [by Skype]*
- 16:25 - 16:50 **Ramesh Hire:** *Distribution and Genetic Diversity of Tomato leaf miner, Tuta absoluta in India*
- 16:50 - 17:30 **DISCUSSION**
- 17:30 - 18:00 Return to hotel

Tuesday, 5 March 2019

SESSION 3 LEPIDOPTERA PRESENTATIONS (CONTINUED)

- 08:30 - 09:00 Walk to Hotel Carillon
- 09:00 - 09:25 **Shiva Osouli:** *Helicoverpa armigera (Lep: Noctuidae): Study of mass rearing, radiobiology and competitiveness ability of parental and F1 generations, in Iran*
- 09:25 - 09:50 **Muhammad Zahid:** *Effect of gamma radiation and chilling temperatures on egg parasitoid, Trichogramma chilonis (Ishii) & Angoumois grain moth, Sitotroga cerealella (Oliv.)*

SESSION 4 OBSERVERS' PRESENTATIONS

- 09:50 - 10:10 **Diego Segura:** *Enhancement of sterile male's sexual competitiveness through hormonal treatment, semiochemicals and gut microbiota management*
- 10:10 - 10:30 **Guillermo Cortes:** *Present Drosophila suzukii situation in the COSAVE region and Argentina. Drosophila suzukii control in Argentina.*
- 10:30 - 10:45 **COFFEE**
- 10:45 - 11:05 **Gastón Crauchuk and Gustavo Taret:** *Agroecology and Biological Control in Mendoza*
- 11:05 - 11:25 **Claudia Conte:** *A molecular and genetic approach to study the interaction between a parasitoid wasp and its host species*
- 11:25 - 11:45 **Sylvia Lanzavecchia:** *Genetics and genomics applied to the study of pest species and beneficial insects*
- 11:45 - 12:05 **Simon Fellous:** *Love, war and transmission: the ecology of Drosophila-microbe symbioses.*
- 12:05 - 13:05 **LUNCH**

SESSION 5 GENERAL DISCUSSION OF THE LOGICAL FRAMEWORK

- 13:05 - 14:45 General Discussion of the Logical Framework
- 14:45 - 15:00 **COFFEE**

SESSION 6 WORKING GROUP DISCUSSIONS

- 15:00 - 15:15 Selection of working group Chairs and Rapporteurs:
1. *Drosophila suzukii*
2. *Spodoptera/Helicoverpa* and *Tuta absoluta*
- 15:15 - 17:30 Working Group Discussions, planning and coordinating work programmes
- 17:30 - 18:00 Return to hotel

Wednesday, 6 March 2019

SESSION 6 WORKING GROUP DISCUSSIONS (CONTINUED)

- 08:00 - 08:30 Walk to Hotel Carillon
- 08:30 - 10:30 Working Group Discussions, planning and coordinating work programmes
(continued)
- 10:30 - 10:45 **COFFEE**
- 10:45 - 12:00 Working Group Discussions, planning and coordinating work programmes
(continued)
- 12:00 - 13:00 **LUNCH**
- 13:00 - 15:00 Working Group Discussions, planning and coordinating work programmes
(continued)
- 15:00 - 15:15 **COFFEE**
- 15:15 - 17:30 Working Group Discussions, planning and coordinating work programmes
(continued)
- 17:30 - 18:00 Return to hotel

Thursday, 7 March 2019

SESSION 6 WORKING GROUP DISCUSSIONS (CONTINUED)

- 08:00 - 08:30 Walk to Hotel Carillon
- 08:30 - 10:30 Working Group Discussions, planning and coordinating work programmes
(continued)
- 10:30 - 10:45 **COFFEE**
- 10:45 - 12:00 Working Group Discussions, planning and coordinating work programmes
(continued)
- 12:00 - 13:00 **LUNCH**
- 13:00 - 15:00 Drafting working group reports
- 15:00 - 15:15 **COFFEE**
- 15:15 - 17:30 Drafting working group reports
- 17:30 - 18:00 Return to hotel

19:00 - 22:00

GROUP DINNER at CLUB MENDOZA DE REGATAS
hosted by
ISCAMEN, MINISTERIO DE ECONOMÍA INFRAESTRUCTURA Y ENERGÍA
DE LA PROVINCIA DE MENDOZA, GOBIERNO DE LA PROVINCIA DE
MENDOZA

Friday, 8 March 2019

SESSION 6 COMPILING RCM REPORT

08:00 - 08:30 Walk to Hotel Carillon

08:30 - 10:30 Reports of Working Groups

10:30 - 10:45 **COFFEE**

10:45 - 12:00 Drafting of RCM report

12:00 - 13:00 **LUNCH**

13:00 - 15:00 General discussion

15:00 - 15:30 **Closing**

15:30 - 16:00 Return to hotel

ANNEX 3 (WORKING GROUPS)

Spodoptera/Helicoverpa/Tuta	<i>Drosophila suzukii</i>
Susana Izquierdo	Simon Fellous
Shiva Osouli	Anna Malacrida
Muhammad Zahid	Lloyd Stringer
Ramesh Hire	Fabiana Sassu
Cynthia Cagnotti	Gustavo Taret
	Alexandra Krüger
	Victor Gutierrez
	Annabelle Firlej
	Allan Debelle
	Simone Puppato
	Emilio Hernández Ortiz

ANNEX 4 (ABSTRACTS OF PRESENTATIONS)

DEVELOPMENT OF MASS REARING AND QUALITY CONTROL PROTOCOLS FOR DROSOPHILA SUZUKII

AUTHOR (S): Fabiana Sassu

ORGANIZATION: Insect Pest Control Laboratory, IAEA, P.O. Box 100, Vienna International Centre, 1400 Vienna, Austria.

SHORT SUMMARY OF PAPER

Abstract:

We assess the effect of different radiation doses on sterility levels in female and male *D. suzukii* under normoxia and hypoxia conditions, and to evaluate the quality of the treated *D. suzukii*. Treating pupae with a dose of 220 Gy under hypoxia resulted in 98% and 100% sterility in male and female adults, respectively without adverse effects on emergence and flight ability when compared with non-irradiated insects. For the mass-rearing two different systems for the artificial collection of eggs of *D. suzukii* were developed. Results showed that wax panel oviposition system have a better performance and it's the most practical method. Therefore, the wax panel can be adopted as the system for mass production of *D. suzukii* for Sterile Insect Technique application.

RECENT ADVANCES IN SIT FOR DROSOPHILA SUZUKII IN CANADA

AUTHOR (S): Annabelle Firlej

ORGANIZATION: IRDA

SHORT SUMMARY OF PAPER

Abstract:

Since July 2017, research on *Drosophila suzukii* SIT focused on 1) the evaluation of competitiveness in laboratory and field, 2) the impact of temperature on adult emergence and deformities and 3) the circadian rhythm of adult emergence. We evaluated the competitiveness of sterile males at different radiation doses in laboratory and field cages; doses tested were 80-90-100-120 grays. In both experiments, among dose treatments, we compared combinations when 10 females were offered 20 sterile males, 20 fertile males or 10 sterile + 10 fertile males. For laboratory, we recorded egg hatching for 5 days and for field cages, we recorded the number of fruits infested and progeny produced. Results in the laboratory showed that sterilized males from 80 to 100 grays are mostly as competitive as fertile males but sterilized males at 120 grays showed decreased in competitiveness. In the presence of only irradiated males at 120 grays, sterility of eggs reached 98.1%. Results in field cages showed no reduction of fruit infested in competition treatment whatever the dose used even if progeny was reduced by 50% with 80 and 120 grays. In the presence of only irradiated males at 120 grays, percentage of infested fruits was 1.7%. In second objective, we evaluated the effect of temperature (10, 15, 25, 30 and 35°C) on the emergence, survival and malformation of irradiated male at 80, 90, 100 and 120 grays. Results are partially available, and we observed that emergence was reduced to 68% for 10 degrees and to 7.6% for 35 degrees. In third objective, we evaluated the circadian rhythm of emergence of drosophila and flight capacity of sterile males. When pupae are irradiated at 80 and 120 grays, adults emerged mainly the morning following 48h after pupae irradiation. Flight capacity experiment results were not positive due to problem in the set-up of the experiment.

IMPLICATIONS OF STERILITY OF DROSOPHILA SUZUKII ON MATING AND SURVIVAL AT DIFFERENT TEMPERATURES AND HUMIDITIES

AUTHOR (S): Alexandra P. Krüger, Dori E. Nava, Flávio R. M. Garcia

ORGANIZATION: Universidade Federal de Pelotas, EMBRAPA Clima Temperado

SHORT SUMMARY OF PAPER

Abstract:

In the literature, there is few information about the mating strategies used by *Drosophila suzukii*, although it is crucial to understand mating and remating occurrence, since it can affect directly the efficiency of Sterile Insect Technique. It is also important to verify mating and survival in different conditions before considering the release of sterile insects. During the past 18 months, we investigated the effects of male and female sterilization on mating and remating behavior and the effects of different temperatures and humidities on survival of both sterile and fertile *D. suzukii*. First, we tested the occurrence of multiple mating in different combinations between sterile and fertile males and females, during a period of 16 days. Then, we tested the effects of male and female sterility on female propensity to mate and remate. We also tested different temperatures (10, 15, 25, 30 and 35°) and humidities (0-20, 21-40, 41-60, 61-80 and 81-100%), by exposing sterile and fertile adults to those conditions, and evaluating for 10 days the survival of the insects and then, the presence of sperm mass in female spermathecae. We found an overall low remating rate by *D. suzukii* females. Male sterility did not influence mating and remating likelihood; however, copula duration of sterile males was shorter compared to fertile males. On the other hand, sterility of the females affected mating probability, and sterile females were less likely to mate. Female sterility also affected latency period, and sterile females took a longer period to start mating, but it did not affect remating probability, duration of copula nor refractory period. There was no effect of radiation on insects' survival in the temperatures and humidities tested, but both conditions affected the overall survival of the insects. Insects were able to survival the 10 days period only when exposed to temperature lower than 30°C humidity higher than 61-80%. The data regarding the presence of sperm mass on female spermathecae was not yet analysed. Although the results found give a glance on mating and remating of sterile and fertile *D. suzukii*, it is important to deeper study remating occurrence in *D. suzukii* and its implications on fertility recovery, also, both temperature and humidity are challenging for *D. suzukii* and must be taken under consideration for release.

**PRELIMINARY ASSESSMENTS TOWARDS THE IMPLEMENTATION OF STERILE
INSECT TECHNIQUE: BASELINE EXPERIMENTS FOR EVALUATING SPERM
UTILIZATION IN DROSOPHILA SUZUKII**

AUTHOR (S): S. Puppato¹, A.R. Malacrida², M. Cristofaro³, G. Anfora¹, C. Ioriatti¹
ORGANIZATION: ¹FEM, S. Michele all'Adige (Trento, Italy)
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SHORT SUMMARY OF PAPER

Abstract:

Little information is available on the reproductive behaviour of *Drosophila suzukii*. This critical point needs to be clarified for the implementation of SIT. In order to improve our knowledge, we carried out mating/remating experiments in which all combination of first- and second-male were taken into account, according to seven crossing types. The trials involved wild-caught, laboratory irradiated and non-irradiated males and females. Over the course of our trials we evaluated copula duration, fecundity and fertility after mating/remating. The most interesting finding arose from experiments with wild-caught *Drosophila suzukii* females, in which we noticed high fertility regardless of the status of their eggs, fertilized or unfertilized, that is crossing with wild-caught fertile or irradiated males, respectively. The results showed that *Drosophila suzukii* females have the ability to select sperm from previous males over recently introduced ones. Genotyping analysis has supported the preliminary hypothesis.

MARKERS FOR ASSESSING THE MATING BEHAVIOUR OF DROSOPHILA SUZUKII

AUTHOR (S): Malacrida AR^R, Puppato S², Gomulski LM¹, Scolari F¹, Di Cosimo A¹

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SHORT SUMMARY OF PAPER

Abstract:

One of the constrains for SIT application is the presence and extent of polyandry.

We have identified a set of informative markers (SSRs) and we have evaluated their suitability for detecting polyandry and sperm use.

With these markers we have identified:

- The presence of polyandry in a population from North Italy
- females use the sperm from all mates

**STERILE INSECT TECHNIQUE AND BIOLOGICAL CONTROL IN CONFINED
CONDITIONS AGAINST *D. SUZUKII* IN MEXICO: PRELIMINARY ADVANCES.**

AUTHOR (S): Víctor Manuel Gutiérrez Palomares

ORGANIZATION: SENASICA

SHORT SUMMARY OF PAPER

Abstract:

Different doses of gamma radiation (0, 60, 70, 80, 90, 180 and 200 Gy) were evaluated on 5-day old pupae of *D. suzukii* for the quality parameters (Emergence, Sexual ratio, emergence time, longevity time, fertility recovery and external morphometry). No significant differences were observed between treatments and control in parameters of (Emergence, Sexual ratio, emergence time, longevity time) in fertility significances differences were observed between the treatments and control on female fertility recovery when a fertile female mates with and irradiated male and re-mates with a fertile male, but it was not immediately. For external morphometry there was not clear tendency to relate the increase or decrease in the external size of the insect with the absorbed dose of irradiation.

The fungus strains CHE-CNRCB 307 of *Isaria javanica*, CHE-CNRCB 224 of *Metarhizium anisoplae* and CNRCB 168 of *Beauveria bassiana* will be evaluated sprayed at different concentrations in adults of *D. suzukii* for measuring the lethal concentration and lethal time. The assessment is in process with the quality parameters of the fungus strains.

Three different markers from the sexual chromosome of *D. suzukii* were amplified and a characterization of haplotypes was done. The haplotypes are similar from the population from USA and Japan.

**MASS REARING, QUALITY PARAMETERS AND FEED CONVERSION IN
DROSOPHILA SUZUKII (DIPTERA: DROSOPHILIDAE), AND THEIR
ECONOMICS ASPECTS**

AUTHOR (S): Marysol Aceituno-Medina¹, Alicia Ordoñez Ruiz², Morfa Carrasco², Pablo Montoya¹, Emilio Hernández¹

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SHORT SUMMARY OF PAPER

Abstract:

The objective of this work was to estimate the mass rearing, quality parameters and feed conversion in *Drosophila suzukii* (Diptera: Drosophilidae), and their economics aspects for sit. The mass rearing, quality parameters and feed conversion in *D. suzukii* and their economics aspects for sit were determined according to a one-way design, in which the treatments corresponded to the diet, corn cob fractions/ torula yeast, corn cob fractions/ brewer's yeast, coconut fiber/ torula yeast, and coconut fiber/ brewer's.

Result. A cage for adults was developed using the cages for the longevity test of sterile *Anastrepha* spp. males. The mass rearing colony was established in the Moscafrut Facility. Three larval diets were developed: Coconut fiber + Torula yeast, Coconut fiber + brewer's yeast, and Corn con fractions + brewer's yeast. The average transformation from eggs to adult was 25%. The feed conversion index was including as mass rearing parameter to evaluate the economic feasibility of the mass rearing, which indicate a low conversion of 2% with a pupal weight of 1.8 mg, 80% of adult eclosion and a yield of 12 larvae per gram of diet.

SAMPLING FOR DROSOPHILA SUZUKII

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SHORT SUMMARY OF PAPER

Abstract:

The sampling efficacy of a *Drosophila suzukii* (SWD) lure & trap combination (scentry lure plus red sticky panel) was estimated and the probability of detecting flies from populations of different densities was predicted. It was estimated that the daily probability of detecting one fly from a population of 50 trappable flies nears 1 once trap density reaches 100 traps per hectare. The probability of detection varies with changes in fly and/or trap density.

An estimate was predicted for the likely population size based on catch of a specific number of SWD in trap(s) on one day when a grid efficacy is known. Incorporation of zero catch and catch over multiple days needs to be added to reduce uncertainty values. Work is still on going in this area.

A sex and age-structured discrete time model to simulate the effects of the different tools on population growth was developed in excel. Using published data, the probable effects of two types of mass trapping and SIT on population growth rates for tools used separately and used together was modelled to predict the effects of these tools on population growth rates. The mass trapping examples were the commercially available scentry lure/red sticky panel trap for SWD and GF-120 protein bait. Based on current tool efficacy, only SIT use is predicted to lead to negative population growth rates. When tools use was modelled in combination, SIT was still the major contributor to population growth reduction.

Further improvements to trapping systems will support outcomes. Revising and validating SIT and the addition of biological control agent estimates based on information generated by participants in the CRP will improve estimates.

**DOES PARTHENOGENETIC REPRODUCTION EXIST IN POPULATIONS OF THE
LEAFMINER TUTA ABSOLUTA (LEPIDOPTERA: GELECHIIDAE) FROM
ARGENTINA?**

AUTHOR(S): Cagnotti C.¹, Conte C.², Lanzavecchia S.², Andorno A.¹ and López S.N.¹
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SHORT SUMMARY OF PAPER

Abstract:

Tuta absoluta is a South American lepidopteran that is considered a main pest of tomato. Our objectives were: to evaluate the capacity of *T. absoluta* populations from Argentina to reproduce parthenogenetically and to detect the presence of *Wolbachia* associated to reproductive abnormalities in this species. Larvae collected from commercial greenhouses of La Plata, Buenos Aires province and from Bella Vista, Corrientes province (Argentina) were reared on tomato plants in the Insectario de Investigaciones para Lucha Biológica IMYZA, INTA (Castelar, Argentina). To study parthenogenetic reproduction in both *T. absoluta* populations, 21 females from La Plata population were isolated in Petri dishes with a tomato leaf during whole life. Also, 19 pairs were left together in Petri dishes under the same conditions. The number of eggs laid and the proportion of emerged larvae were recorded. In Bella Vista population, 20 females alone and 20 pairs were evaluated. Fecundity was significantly higher for mated females compared to unmated ones in both populations. In La Plata population, unmated females laid an average of 4 non-fertile eggs/female while mated females laid 48 eggs/ female with a fertility rate of 0.73. In Bella Vista population, unmated females laid an average of 5.05 non-fertile eggs per female and, in the pairs, females laid an average of fertilized 94 eggs/female with a fertility rate of 0.76. Besides, we amplified and sequenced the 16SrRNA gene from adults of La Plata population and the results indicated 100% *Wolbachia* prevalence in *T. absoluta* individuals and its sequencing was 100 % identical to the *Wolbachia* of other lepidopteran species.

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

AUTHOR (S): Carolina Yanez, David Castro, Susana Izquierdo, Viviana Pavez and George Saour

ORGANIZATION: Foundation for the Development Fruticola

SHORT SUMMARY OF PAPER

Abstract:

The first tests of irradiation with 200 and 150 Gy were made with the biological material collected in the field, in both cases, the sterility index obtained was very low, F1 was obtained, but it obtained a very weak development. The average longevity of males and females is not altered by these doses.

The sterility index was calculated based on the fecundity and fertility of the treatment with respect to the control.

A very important point is that these crosses are only from the same mother. All combinations, however, are still very fertile. The sterility index of the F1 with male and / or wild female will be evaluated in the next 18 months. New wild insects are incorporated to avoid inbreeding.

Massive breeding is considered of great importance due to the homogeneity of the biological material.

Four diets were tested and tomato plants were used as control. The biological cycle is completed in one of the 4 diets tested. Currently, we are working on its improvement.

To consider which would be the best option, 4 parameters were taken as reference. The first was evaluated at 5 days of development and corresponds to the percentage of larval mortality. Diet 1 was the one that obtained the lowest percentage mortality, with respect to the control (43.4%).

The second parameter corresponds to the percentage recovery of pupa, from 50 larvae of L3, where the best option continues being Diet 1, with 20.7%. This value is still very low, so, we work on improving the diet.

The first exploratory tests in *Tuta absoluta* were performed with *Goniozus legneri* and *Trichogramma nerudai*, the initial results are positive for the start of the tests in each biological controller.

**EVALUATION OF SIMULTANEOUS TREATMENTS OF STERILE INSECT
TECHNIQUE AND BIOLOGICAL CONTROL AGENTS FOR SPODOPTERA
LITTORALIS MANAGEMENT**

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SHORT SUMMARY OF PAPER

Abstract:

The cotton leaf worm, *Spodoptera littoralis* (Boisd.) is a highly polyphagous defoliator of many cultivated plants with a wide range of hosts, feeding on 112 species worldwide. Six larval diets based on the modifications of the semi artificial diet developed by Shorey and Hale were tested in addition with the castor oil plant in the hope of providing an economically viable diet for *S. littoralis* mass rearing. Out of the six diets, diet based on castor leaf powder was most suitable for *S. littoralis* rearing followed by diet based on white kidney bean and brewer's yeast. Castor leaves for rearing the culture are not available throughout the year.

For the simultaneous treatment of SIT/IS with baculovirus, neonate *S. littoralis* larvae were treated with 1×10^3 PIBs died in the 5th instar and led to highest yield of NPV virus. The results obtained showed that the two doses 40 and 60 Gy are effective doses for irradiating *S. littoralis* male pupae to produce F1 larvae very sensitive to SpliNPV.

The dose of 125Gy in combination with 50 and 100 ppm Bt protein resulted effective control of *S. littoralis*.

The combination study of SIT/IS and *Chrysoperla carnea* results showed that the 2nd instar larvae of *C. carnea* prefer the un-irradiated eggs of *S. littoralis* than that irradiated with gamma irradiation (100, 130 and 160 Gy) and also the lower doses (100Gy) more preferred than the high 130 and 160 Gy.

**DISTRIBUTION AND GENETIC DIVERSITY OF TOMATO LEAF MINER TUTA
ABSOLUTA POPULATIONS IN INDIA IN RELATION TO STERILE INSECT
TECHNIQUE APPLICATION**

AUTHOR (S): Ashok B. Hadapad, V. P. Venugopalan and Ramesh S. Hire
ORGANIZATION: Nuclear Agriculture and Biotechnology Division, Bhabha Atomic
Research Centre, Trombay, Mumbai 400085 India

SHORT SUMMARY OF PAPER

Abstract:

The tomato leaf miner, *Tuta absoluta* (Meyrick) is one of the major insect pests of solanaceous crops. It was first reported in India in 2014 and subsequently it has been reported from several states of India. Extensive survey was undertaken during 2017-2019 to collect the wild populations of *T. absoluta* from 11 districts of 8 states of India. The infestation level (40-80%) was recorded in Maharashtra, Karnataka, Telangana, Tamil Nadu, Haryana, and Himachal Pradesh while, infestation was not observed from Eastern Indian states. This indicates that the infestations of *T. absoluta* are more prevalent in Southern and Western states as compared to Northern and Eastern states of India. Phylogenetic analysis using mitochondrial *COI* gene sequences has revealed that nine *T. absoluta* populations are genetically identical as these sequences are grouped under single clade. This suggests that *T. absoluta* has been introduced at a single site and then expanded to different geographical regions of India. The samples were also used for detection of *Wolbachia* using *16S rRNA* analysis and further characterized using Multi Locus Sequence Typing (MLST) and *wsp* approach. Total 75 samples from 9 locations were screened for *Wolbachia* of which 32 were found to be positive. This indicates that the overall 42.66% of tomato leaf miner populations were infected with *Wolbachia*. Based on the phylogenetic and MLST analysis, the prevalent *Wolbachia* strain belong to supergroup B, which is parasitic in nature. The distribution, host range, population dynamics, genetic composition of *Tuta* and importance of *Wolbachia* in *Tuta* management will be discussed.

**HELICOVERPA ARMIGERA (LEPIDOPTERA: NOCTUIDAE): STUDY OF MASS
REARING, RADIOBIOLOGY AND COMPETITIVENESS ABILITY OF PARENTAL AND
F1 GENERATIONS, IN IRAN**

AUTHOR (S): Shiva Osouli, Mehrdad Ahmadi

ORGANIZATION: Nuclear Agriculture Research School, Nuclear Science and Technology
Research Institute

SHORT SUMMARY OF PAPER

Abstract:

Helicoverpa armigera Hübner (Lepidoptera: Nuctuidae), is one of the most important pests in the world which is established all over of Iran, reported in 1938 for the first time. The artificial diets and rearing system have been developed and improved for rearing successive generations of *H. armigera*, which made the effective mass rearing of this pest possible as essential component of SIT. The effects of different doses of gamma radiation in the range of 100 to 350 Gy on some biological parameters of *H. armigera* were evaluated. The mean percentage of pupal mortality increased with increasing doses when 8-day old pupae were irradiated (27.67% at 350 Gy compared to 16% in the control). The lifespan of male and female *H. armigera* irradiated in the pupal stage, were significantly shortened. The number of eggs (fecundity) of the female moths remarkably affected by radiation in all crosses. The percentage of hatched eggs (fertility) reached 0 at 350 Gy in various reciprocal crosses, while this value was less than 4% when normal males mated with irradiated females at 300 Gy. The mean percentage of hatched eggs laid by F1 female progeny markedly decreased when male parents irradiated at 150 Gy and reached 0 at the dose of 200 Gy in both crosses (F1 males or F1 females mated with normal pairs). The sex ratio of emerged adults of F1 progeny from irradiated male parents skewed to male with increasing doses and reached to 0.66 at 200 Gy. Gamma-sterilized male insects and F1 male progeny from sub-sterilized male parents were as competitive as the untreated ones in terms of mating and the calculated competitiveness value of the treated males in both generations was within the acceptable range.

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)

ORGANIZATION: Plant Protection Division, NIFA, P.O. Box-446, Peshawar, (25000) Pakistan.

SHORT SUMMARY OF PAPER

Abstract:

The egg parasitoid, *Trichogramma chilonis* (Ishii) is widely used in Pakistan in biological control programs. An essential aspect in improving the economics of the mass production is to use some means to extend the shelf-life of host eggs to ensure their continuous supply. A variety of options may be used for egg preservation including gamma radiation and cold storage.

Egg parasitoid, *Trichogramma chilonis* (Ishii) production point of view, it is important to use younger adult parasitoid (12-24 h) age and fresh Angoumois grain moth, *Sitotroga cerealella* (Oliv.) eggs (12-24 h) on green colour paper cards to get maximum parasitism for 24 hours of exposure time. Radiation dose (10-20 Gy) is very conducive for maximum parasitism, egg hatching, adult emergence and incubation period (8-9 days) of *Sitotroga cerealella* eggs to get short term storage (3-5 days). Low storage temperatures (4°C) are also very effective for maximum parasitism as well as prolong the incubation period of *Sitotroga cerealella* eggs. Low radiation doses and cold storage increases shelf life of *Sitotroga* and *Trichogramma* without maximum detrimental effects on the quality of egg parasitoid and host. This can ensure the year around availability of parasitoid in insectaries for research and field releases.

A MULTIVARIATE AND ECOLOGICAL FRAMEWORK FOR THE UNDERSTANDING OF HOST-MICROBE SYMBIOSIS

AUTHOR (S): Simon Fellous, Robin Guilhot

ORGANIZATION: INRA-CBGP, Montpellier, France

SHORT SUMMARY OF PAPER

Abstract:

Microbial symbionts can either provide resources to their hosts or mediate host plasticity through physiological signalling. The expected evolutionary fate of host-microbe associations changes whether microbes affect available resources or its plasticity, the effect of the latter being sensitive to environmental conditions.

Here we propose an empirical framework to separate symbiont-induced effects on resource availability and plasticity. We applied the framework to a series of experiments on the symbiosis between bacteria, yeasts and *Drosophila* flies.

Our results show that yeast in *D. suzukii* larvae boosts general performance and affect their developmental plasticity along the trade-off between speed of larval development and adult size. These results can be used to best chose nutritional yeasts for mass-rearing and the production of competitive sterile males.