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WORKING MATERIAL

Integration of SIT with Biocontrol for Greenhouse Insect Pest Management

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

Virtual

21–25 June 2021

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

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

Tuta 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 21–25 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

THIRD RESEARCH CO-ORDINATION MEETING (RCM)

Due to the travel restrictions related with the Covid-19 pandemic the Third RCM was organized as virtual meeting from 21 to 25 June 2021. Fifteen scientists and several observers from 12 countries attended this second RCM. The list of participants, which included CRP contract and agreement holders, as well as 4 additional observers, is given in Annex 1. The agenda for the meeting is attached in Annex 2.

During the first two days of the meeting, RCM participants presented progress of their research relevant to the CRP, as well as their research plans for the next 18 months that will the final face 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 last face of the CRP. Most of the countries were affected by the covid-19 pandemic, and most projects were suspended or restricted, as consequence, some planned activities have been cancelled or postponed. 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
Drosophila suzukii	Gustavo Target Annabelle Firlej	Aikaterini Nikolouli Massimo Cristofaro Gustavo Taret Fabianna Sassu Victor Gutierrez Robin Guilhot Emilio Hernandez	Massimo Cristofaro Anna Malacrida Fabiana Sassu Gustavo Taret Alexandra Krüger Victor Gutierrez Annabelle Firlej Aikaterini Nikolouli Robin Guilhot	Alexandra Krüger Massimo Cristofaro Victor Gutierrez Gustavo Taret Lloyd Stringer	Anna Malacrida Allan Debelle Massimo Cristofaro Gustavo Taret Alexandra Krüger Simon fellous	Massimo Cristofaro Anna Malacrida Fabiana Sassu Gustavo Taret Alexandra Krüger Victor Gutierrez Annabelle Firlej Aikaterini Nikolouli Robin Guilhot
Tuta absoluta	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	
Spodoptera littoralis	Waheed Sayed	Waheed Sayed	Waheed Sayed	Waheed Sayed		
Heliocoverpa armígera	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 pheromome or plant kairomones are unknown.
7. The dynamics of *D. suzukii* populations in greenhouses (sex-ratio, circadian cycle, seasonality…) are not defined.

Radiation biology for *D. suzukii*

Current knowledge:

The dose-response for males and females at the pupal stage in the presence or absence of O_2 (i.e. normoxia/hypoxia) is under evaluation (Lanouette et al. 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 to determine the doses that induce full sterility. Quality control test (longevity, weight of pupae, survival, flight…) should be performed for higher doses than 120Gy, to determine the balance between sterility induction and sterile male fitness. It is crucial to do the characterisation of the sexual competitiveness of irradiated males (mating, flight, dispersion capacity, etc) to select the appropriate doses inducing sterility.
2. Information on sperm viability and sperm competitiveness of irradiated males (morphology, ejaculate size, sperm number, etc) is lacking, as well as the existence of cryptic female choice between irradiated and non-irradiated sperm.
3. Effect of irradiation doses on sperm/ejaculate transfer, refractory period and re-mating frequency is important and unknown.
4. The best time interval for egg collection, as well as the optimal female age, should be evaluated to ensure optimal progeny collection (progeny quality, irradiation effectiveness and genetic variability).
5. Mating compatibility within a strain between irradiated laboratory males and wild females (colonization effect) is unknown, as well as the mating compatibility between strains of different origins.
6. The effects of sterile female oviposition (i.e. skin puncturing) on fruit quality and susceptibility to pathogenic infections have to be evaluated.
7. Impact of hypoxia and temperature before and after irradiation on quality control parameters should be evaluated.

Potential sexing system

Current knowledge:

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

Gaps identified:

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

Mass rearing for *D. suzukii*

Current knowledge:

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

Gaps identified:

1. Synchronisation of pupae emergence should be evaluated to improve the quality of the mass rearing and its cost-effectiveness.
2. The definition of optimal mass-rearing artificial diet(s) for high quality larval and adult performance is lacking.

3. Techniques for egg collection and pupal separation are lacking.
4. Cost analysis of mass-rearing is missing.

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

Current knowledge:

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

Gaps identified:

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

1.1. Standard protocols

Participants: all participants

5 year plan:

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

18 month plan:

- Create a protocol for all teams to perform standardised experiments: traps and baits used for sampling, artificial diet for rearing, dosimetry, voucher sampling frequency.
- Standardize quality control parameters: emergence and flight ability, longevity under stress, mating competitiveness.

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).
- 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 (RCM2 Mendoza, Argentina 2019)

- 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.

Results from the previous 18 months as planned during RCM2

Due to Covid pandemic limitations some planned activities could not to be performed or partially performed

- Results were obtained in relation to the genetic characterization, reproductive biology and presence of polyandry in populations of a North Italian region. We collaborated with researchers of the Edmund Mach Foundation (TN /Itly) to analyze the reproductive dynamics across seasons of *Drosophila suzukii* populations in a north Italian region /Trentino where this pest can overwinter. Here the females are reproductively active from early spring until late autumn, and the most stringent bottleneck for *D. suzukii* populations is from January to March.
- Geographic populations were sampled during winter and summer seasons and compared for demographic parameters (fertility, fecundity, sex ratio) and genetic.
- Structure. Using molecular markers, the presence /levels of polyandry and sperm use have been assessed during the summer period when the population is in the peak of demographic. In this population polyandry is a normal behaviour and its extent is large. The females use the perm from different mates.
- Data on polyandry during the winter period (population bottleneck) is under test.

18 months plan (RCM3 Seibersdorf – Virtual)

With flies from North Italian populations already characterized for the presence of polyandry and other demographic parameters, perform mating and remating laboratory tests to analyse:

the effect of age and male/female density on the degree of polyandry
the temporal use of sperm,
the refractory period (if any)
how polyandry affect the -fecundity, fertility
Define if any the level of paternity skew.
Evaluation of sperm viability in spermathecae in relation to storage time
Use modelling for predicting population dynamics (With Lloyd Stringer)
Assess the reproductive dynamics and presence/degree of polyandry in populations from different geographic origins where the SIT has been planned.

- develop new methods for sperm marking.

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 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).
- Analyses show a limited role of olfactory signals at best, but a significant importance of the dark spot characteristics.
- Selection of a *D. suzukii* line with superior male mating success, validated in field-like cages with wild females.

18 month plan (RCM3 Seibersdorf – Virtual):

- No plans for the next 18 months

Participants: Massimo Cristofaro and Simone Puppato (Italy) (observed)

5 year plan:

- 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).
- 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 (RCM 2 Mendoza, Argentina 2019):

- 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 spermatechae 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).

Results from the previous 18 months as planned during RCM2

- Polyandry has been assessed in a summer Italian population at its peak of density. The polyandry is present, and its extent is high.

18 months plan (RCM3 Seibersdorf – Virtual)

- Evaluate the presence of polyandry in winter populations when the density is low, and the females have experienced reproductive diapause.

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 (RCM2 Mendoza, Argentina 2019):

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

Results from the previous 18 months as planned during RCM2

- Sampling protocols will be applied and introduced in the designed GIS system postpone to the next 18 months.

18 months plan (RCM3 Seibersdorf – Virtual)

- 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 (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 (RCM 2 Mendoza, Argentina, 2019):

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

Results from the previous 18 months as planned during RCM2

- Due to the Covid constrains this activity was cancelled.

18 months plan (RCM3 Seibersdorf – Virtual)

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

Participants: Robin Guilhot 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 (RCM2 Mendoza, Argentina 2019):

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

Results from the previous 18 months as planned during RMC2:

- Competitiveness of flies irradiated under normoxia and hypoxia conditions at the two doses was assessed by Fabiana. A paper will be published soon.
- Additional project: we seeked to compare the effects of Gamma and X-ray irradiation under hypoxia on sterility and quality of the flies mass-reared at the IPCL at the two doses identified by Fabiana for suppression (170 Gy) and eradication (220 Gy). Our data – although uncomplete – show that expected sterility levels are achieved with both technologies for the two doses. In addition, fly quality does not seem to differ between the two technologies for the two doses.

18 months plan (RCM3 Seibersdorf – Virtual)

- Finish comparing effects of Gamma and X-ray irradiation on fly sterility and quality to statute if X-ray irradiation is a good alternative to Gamma irradiation for D. suzukii sterilization.
- Assess the competitiveness of IPCL mass-reared irradiated flies (using one or both radiation sources under hypoxia) using wild fertile flies under several conditions, including field/ semi-field ones.

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 (RCM2 Mendoza, Argentina 2019):

- 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 springtime)
        - Greenhouse (strawberries)
            - Natural conditions (to be performed at the beginning of springtime) in collaboration with Simon and Allan.

Results from the previous 18 months as planned during RCM2:

- Pre and post irradiation test of quality control parameters were evaluated with different doses (70-140-170-180-200 Gy)- Fly ability and survival under stress were evaluated. Statistical significance difference found between fertile flies and sterile flies of different doses. However, no significance difference between the doses.
- Competitiveness fried test were evaluated in 2021 under greenhouses field conditions during four weeks of releasing. From the first releasing to the last releasing the values of competitiveness index increased according adjustment of irradiation time close to emerging.

18 months plan (RCM3 Seibersdorf – Virtual)

- Continue with pre and post irradiation quality control parameters using 140 – 170 - 200 Gy introducing the analyses of offspring after eggs collected from the competitiveness plot (larvae, pupae and adults recovery)
- Continue with competitiveness Fried Test
    - Source of flies
        - Sterile flies from the lab colony
        - "wild flies" kept under natural condition (fruit).
    - Dose to be evaluated: 140 – 170 - 200 Gy.
    - Environment conditions
        - Small plexiglass cages (lab conditions)
        - Field cages
            - Field conditions

- Greenhouse (strawberries)
    - Natural conditions (to be performed at the beginning of springtime) 5 months.

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.

Results from the previous 18 months as planned during RCM2:

- The results from experiments regarding effects of temperature and humidity on longevity and mating of irradiated *D. suzukii* adults were published in Journal of Applied Entomology in 2021.

18 month plan (RCM3 Seibersdorf – Virtual):

- Completed but we plan to include tests on how the presence of sterile females affects the efficiency of sterile *D. suzukii* males.

Participants: Victor Gutierrez (Mexico)

5 year plan:

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

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. suzukii* 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.

18 month plan (RCM2 Mendoza, Argentina, 2019)

- Completed.

Results from the previous 18 months as planned during RCM2:

- **Not completed due the COVID-19** We could only do one repetition for the evaluation of a packing, transporting and release system for sterile *D. suzukii.* With the flies for the first repetition, we carried out an experiment of the potential effects of irradiation at 200 Gy with Cobalt-60, sex and artificial diet based on coconut fiber in wing morphology was analyzed by means of a geometric morphometry analysis. No effect on wing morphology caused by irradiation was observed, however, significant differences were found in the centroid size of wild females compared with irradiated and non-irradiated females developed in artificial diet ($F_{147,2}$ = 67.45, P = <0.0001), but in Procrustes distances they showed no significant difference ($F_{144,2}$ = 0.81, P = 0.4477); wild males showed significant differences in centroid size ($F_{147,2}$ = 59.55, P = <0.0001) and Procruste distances ($F_{144,2}$ = 3.49, P = 0.0330) compared to irradiated and non-irradiated males developed in artificial diet in this sense, changes in the wing of *D. suzukii* are more related to the feeding of insects in immature stages. Published on Southwestern entomologist in 2020.

18 month plan (RCM3 Seibersdorf – Virtual):

- If the Covid-19 situation in México allows it, we will continue with the Packing, transporting and release system for sterile *D. suzukii* in colaboration with Pablo Montoya.

Participants: Annabelle Firlej (Canada) absent no report

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 (No activities repoted)

5 year plan:

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

Results from the previous 18 months:

- Fitness and cytoplasmic incompatibility (CI) experiments was performed for all *Wolbachia* infected and uninfected lines of *D. suzukii*.
- 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.

Participants: Simon Fellous and Clelia Oliva (France)

5 year plan:

- Developing an efficient and flexible solution for *D. suzukii* sterilisation.

Results from the previous 18 months:

- Experiments with X-ray sterilisation of D. suzukii pupae were successful.

18 month plan (RCM3 Seibersdorf – Virtual):

- Attempt at sterilizing young adults rather than pupae.

1.4. Mass rearing for D. suzukii

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

5 year plan:

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

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 (RCM2 Mendoza, Argentina 2019):

- 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. suzukii* (pupae or adults) (collaborate/communicate with IPCL).

Results from the previous 18 months as planned during RCM 2:

- A new diet on carrot fibre was implemented for the mass rearing of *D. suzukii.*
- A new disposal for the flight ability test was evaluated.
- The optimal age at which the pupae should be irradiated at 200 Gy was established, quantifying the percentage of eyes colour of pharate adults from 4-day-old pupae.

- The evaluation of packing and transporting of sterile *D. suzukii* pupae was initiated (1 repetition), but suspended by COVID pandemic cause.

Additional 18 months plan (RCM3 Seibersdorf – Virtual)

- To complete the test of sterility induction into wild populations at laboratory and semifield level
- To complete the evaluation of packing and transporting of sterile *D. suzukii* pupa, in collaboration with Victor Gutierrez.

Participants: Aikaterini Nikolouli and Konstantinos Bourtzis (Vienna)

5 year plan:

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

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) absent no report

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. suzukii*.
- Develop a system for packaging and releasing sterile *D. suzukii* (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 (RCM2 Mendoza, Argentina 2019):

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

Results from the previous 18 months as planned during RCM2:

- To develop egg collection and pupal separation technique.
    - Eggs collection system using jelly substrate optimize the eggs collection however it should be improve and reduce the handling.
- Measure colonisation effects in laboratory rearing.
    - The evaluation during 5 generations of colony strain respect of wild strain showed significant difference on % emergence and survival.

18 months plan (RCM3 Seibersdorf – Virtual)

- Evaluate the effect of centrifugal prototype machine on the quality parameters specifically fly ability, efficiency of machine and survival of pupae before and after machine.
- Measure colonisation effects in laboratory rearing during 10 generations comparing fly ability, survival and compatibility test against wild flies.

Participants: Fabiana Sassu, Robin Guilhot 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 (RCM2 Mendoza, Argentina, 2019):

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

Results from the previous 18 months as planned during RCM2

- Paper accepted for publication.

Additional 18 months plan (RCM3 Seibersdorf – Virtual)

- Test different larval/adult diets and probiotics (yeasts and bacteria) to modulate fly production and quality (in collaboration with Simon Fellous).
- Publish an IAEA document that details the IPCL-developed mass-rearing method for *D. suzukii.*

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

Participants: Pablo Montoya, Rubén Hernández, Marysol Aceituno-Medina, Emilio Hernández (México), in colaboration with Víctor Manuel Gutiérrez Palomares (México).

5 year plan:

- To evaluate the horizontal transmission of entomopathogenic fungi between sterile and fertile flies.
- **Additional 18 months plan (RCM3 Seibersdorf – Virtual)**
- To evaluate the horizontal transmission of entomopathogenic fungi between sterile and fertile flies.

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 (RCM2 Mendoza, Argentina 2019):

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

Results from previous 18 months as planned during RCM2

- We performed experiments regarding the release of the parasitoid *Trichopria anastrephae* in greenhouses, and found that releasing a 1:6 ratio (*D. suzukii: T. anastrephae* ratio) can decrease *D. suzukii* population by 66.38%.

18 month plan (RCM3 Seibersdorf – Virtual):

- We will test joint releases of sterile *D. suzukii* and *T. anastrephae,* to verify the impact on *D. suzukii* population.

Participants: Massimo Cristofaro and Simone Puppato (Italy) absent no report

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 (RCM 2, Mendoza, Argentina 2019):

- A bioassay with fungal strains. Concentration of $1X10^8$ sprayed on *D. suzukii* adult bodies to measure lethal time.
- Run treatments with 4 concentrations of the fungus $1X10^6$-$1X10^9$ sprayed on *D. suzukii* adult bodies to measure lethal concentration and lethal time.

Results from the previous 18 months as planned during RCM2:

- Completed. The evaluated strains showed different degrees of pathogenicity and virulence on the adults of *D. suzukii*. The strain of *I. javanica* (CHE-CNRCB 307) was the one that registered the largest significant differences with an LC_{50} of $1.6x10^4$ adult conidia per ml-1 and a LT_{50} of 4.579 days. The strains of *M. anisopliae* (CHE-CNRCB 224) and *B. bassiana* (CHE-CNRCB 168) were not significantly different when compared with each other with an LC_{50} of $3.1x10^5$ and $1.4x10^6$ adult conidia per ml^{-1} and LT_{50} of 18.16 and 15.3 days respectively. The manuscript has sent for publication.

18 month plan (RCM3 Seibersdorf – Virtual):

- Complete the manuscript and participation in Agronomic congress in Mexico.
- Test horizontal transmission between sterile insects and fertile insects, in collaboration with Pablo Montoya.

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 (RCM2 Mendoza, Argentina, 2019):

- 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

Results from the previous 18 months as planned during RCM2:

- Postpone to the next 18 months.

18 months plan (RCM3 Seibersdorf – Virtual)

- Bioassay under greenhouses under natural conditions next spring (2021), checking level of damage on the fruit when management tools are used; Seasonal time (approx. 3 months).
    - SIT
    - SIT+ BC (*Dicyphus sp)*

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 (RCM2 Mendoza, Argentina 2019)

- 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.

Results from previous 18 months as planned during RCM2

- Using new published data, revised the estimate for the probability of catch based on the efficacy of the trap and the number of traps in an area.
- Updated population growth model with data published by participants and others.
- Began to estimate the effect of insect biological control agents (BCAs) on SWD population growth rates.
- Investigated the combination of BCAs and sterile insects on expected population growth rates.
- Started developing an excel spreadsheet model that could be used by growers or their support industries to estimate population density for subsequent sterile fly and/or BCA release rates.

18 month plan (from virtual meeting)

- Work with participants to finalise all modelling.
- Complete the tool a grower or their support industry could use to support SWD management.

Participants: Simon Fellous and Clelia Oliva (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 degredation (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 parametres 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. Artifical 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)

5 year plan:

- Develop standard protocols for infestation level assessment.
- Establishing and maintaining *T. absoluta* colony in the laboratory.
- Studying the possibility of rearing *T. absoluta* on artificial diet.
- Studying the quality control parmeters of reared insect.
- 11%.

18 month plan:

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

Results of the 18 months as planned in RCM2

- **Establishing and maintaining *T. absoluta* colony in the laboratory:**

During the first stage of the project, the breeding of *Tuta Absoluta* in tomato plants was established. At the same time, work begins on point 2, maintaining the support of biological material in the tomato plants.

- **Maintained breeding of *T. absoluta* with artificial diet:**

The selected diet was kept under evaluation.

- **Validating quality parameters of artificial breeding:**

Among the quality parameters evaluated; the survival percentage of larvae, pupal formation and adult emergence are the most representative.

Next 18 month plan:

- Economic evaluations of diets and prices per kilo.

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

5 year plan:

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

18 month plan:

- 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.

Results of the past 18 months

- Effectiveness four larval artificial diets based on used commercial sources of gelling components as substitute of an expensive ingredient (agar) alongside the castor leaves diet was assessed.
- the pupal and adult recoveries produced by castor bean leaves were significantly high followed with agar-based diet. Moreover, the larval durations were significantly prolonged for (starch+ gelatin)-based diet compared the other dietary diets.
- Comparative biochemical studies have shown that there are negligible variations between different diets.
- The Baculovirus (*Spli*NPV) pathogenicity against the larvae reared on (starch+ gelatin)-based diet was 2.5 and 2 times higher than those reared on castor leaves and agar-based diet, respectively.
- the sterility doses of male moths produced by castor leaves and agar-based diet were relatively similar and could be considered as a promising choice for SIT program.

Next 18 month plan:

- Developing the rearing technique of *S. littoralis* on semi artificial diet.
- Maintaining the colony of *T. absoluta* under laboratory conditions.
- Continually the attempts for rearing methods of *T. absoluta* on semi artificial diet.

Participants: Ramesh Hire and Ashok Hadapad (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.

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.*

Results from the previous 18 months as planned in RCM2

- *T. absoluta* was more prevalent in Southern and Western states of India as compared to Northern and Eastern states.
- The *mtCOI* gene sequences of *Tuta* samples showed no genetic variation with high genetic homogeneity.
- Mass rearing of tomato leaf miner culture was optimised on tomato seedlings in insect rearing and cloth cages resulting in continuous supply of different stages for experiments.

Next 18 months plan

- Continue to optimise mass-rearing protocols for *T. absoluta* on identified artificial diets.

Participants (Muhammad Zahid, Usman Khalique, Noor Fatima & Syed Jawad Ahmad Shah (PAKISTAN)

5 year plan:

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

18-month plan (RCM2 Argentina):

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

Results of the last 18 months (RCM3 Virtual meeting)

- *Trichogramma* pupae preservation point of view, it is important to use radiation upto 5.0 Gy for maximum adult emergence (83.0%) & longevity (7.33 days).
- Low storage temperature (2°C) is very effective for maximum *Trichogramma* adult emergence (84.67%) & longevity (7.33 days) for 3 days stored pupae.
- Low radiation and cold storage increase shelf life of *Trichogramma* pupae without maximum detrimental effects on quality of parasitoid during transportation and ensure year around availability in insectary for research & field releases.

- The tomato plot (48 m2) treated with 1500 *Trichogramma* pupae was less infested by fruit worm i.e., 0.41/plant followed by 1000 pupae (0.67/plant), 500 pupae (0.85/plant) as compared to control plot (1.30/plant). Okra production was also higher in 1500 pupae/plot i.e., 32.88 Kgs. followed by 1000 pupae (27.46 Kgs.), 500 pupae (22.33 Kgs.) as compared to untreated plot (17.75 Kgs.).
- The okra plot (40 m2) of each treated with 1500 *Trichogramma* pupae was also less infested by fruit worm upto 0.35/plant followed by1000 pupae (0.63/plant), 500 pupae (0.77/plant) as compared to control plot (1.13/plant). Okra production was also higher in 1500 pupae/plot i.e., 24.25 Kgs. followed by 1000 pupae (20.50 Kgs.), 500 pupae (17.88 Kgs.) as compared to untreated plot (13.29 Kgs.).
- Maximum population of fruit worm moths were recorded in April i.e., 20.50/trap followed by March (6.75), Sept. (4.50), July (3.88), May (2.50), Nov. (2.00), June (1.75), Oct. (1.25), Aug. (0.50) & no moth was caught in Dec., Jan. & Feb. (2019).
- Minimum infestation of fruit worm, *H. armigera* & okra shoot/ fruit borer, *E. vitella* was recorded i.e., 0.20 & 0.50/ plant in *Trichogramma* (2000 nos.) treated okra plot (24 m2) followed by 1500 pupae (0.27 & 0.58/plant), 1000 pupae (0.32 & 0.67) & maximum in control plot (no. pupae card) i.e., 0.40 & 0.92/ plant respectively in high tunnel okra from July- November (2020). Okra plot treated @ 1500-2000 *Trichogramma* was found less affected by borers as compared to control.
- Maximum parasitism of *Sitotroga* eggs by *Trichogramma* were recorded upto (33-43%) at a distance of 2-4 feet whereas minimum parasitism (7-22%) at a distance of 6-10 ft. in tomato pots in glasshouse. Maximum *Trichogramma* adults were emerged upto 80-93% from these parasitized eggs when brought into bio-control lab. So, parasitism is inversely proportion to distance covered.
- During biological study of fruit worm, *Helicoverpa armigera* (Hub.) insectary improvement on different artificial/ natural diets which showed that the low-cost artificial diet-A (chickpea flour base) along with other essential diets ingredients was found very effective to get good quality culture of fruit worm as compared to artificial diet-B (maize flour base) and control diet-C (natural chickpea).

Training workshops conducted during (2019–2020)

1. One day training workshop on "Bio-control of insect pests of vegetables, field crops & fruit orchards" on April 19, 2019 held at Naguman farm center, Peshawar, Pakistan.
2. One day National farmer's awareness seminar on "disease & insect pests of apple" on September 05, 2019 held at Kalam sub-station, Mingora, Swat, Pakistan.
3. One day training workshop on "Popularization of insect pests control technologies for commercialization" on March 11, 2021 held at Agric. Services Academy (ATI) Peshawar, Pakistan.

Next 18-month plan (RCM3 Virtual)

- To maintain bio-control insectary for egg parasitoid, *Trichogramma chilonis* (Ishii) on host eggs of *Sitotroga cerealella* (Oliv.).
- Host preference studies on the life parameters of Angoumois grain moth, *S. cerealella* on different stored cereals.
- Laboratory evaluation of different host diets on the fitness of *T. chilonis*.
- To evaluate the parasitizing potential of *T. chilonis* with bio-pesticides against fruit worm, *Helicoverpa armigera* (Hub.) in high tunnel tomato.

- To compare natural and artificial diets for effective rearing of fruit worm, *H. armigera* in vitro condition.
- Determining the sterilizing and sub-sterilizing doses of *H. armigera*.
- Ecological study of tomato fruit worm, *H. armigera t*hrough pheromone baited traps.
- Dissemination of bio-control technology through training workshops, pamphlets and radio talks among progressive farmers.

Participants (Shiva Osouli, Mehrdad Ahmadi (IRAN)

5 year plan:

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

18 month plan:

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

Results of the previous 18 months

- Mass rearing on artificial diet and monitoring the quality of laboratory-reared *H. armigera* have continued.

Next 18-month plan (RCM3 Virtual)

- 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 successefully manged by gamma irradiation in many regions around the world. Inherited sterility (IS) offers significant advantages over classical sterile released method for lepidopteran pests. The IS technique depends on using substerlizing doses of gamma radiation, these doses induce deleterious effects that are inherited by the F1 generation. IS was employed in many regions to eradicate Lepidopteran pests.

The objectives can be summerized as follow:

1. 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. absoulta* (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)

5 year plan:

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

18 month plan:

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

Results of the 18 months as planned in RCM2

Radiate pupae male and female *T. absoluta* with 100, 130, 150 and 200 Gy doses:

- The 150 Gy dose was selected because no significant difference was detected with the 200 Gy dose.
- Just to validate, new tests will be carried out with 200 Gy using moths from artificial diet. This activity will be carried out before the release in field cages of SIT with biological controllers.

Evaluating the viability of the radiated offspring (F1).

- When only the male is irradiated, a sterility index close to 31% is achieved with a viable F1. However, when it is only the irradiated female, it has a sterility index of 55% but only 13% reaches the pupa.

18 month plan:

- To conduct a competence test (This test will determine the numerical relationship for suppression in the laboratory).

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.

18 month plan:

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

Results of the past 18 months

- Male and female pupae of *S. littoralis* were exposed to 5 different doses (150, 175, 200, 225, 250 and 300 Gy) Moreover, the competitiveness of irradiated and unirradiated males for mating with un-irradiated females (UI♀) was studied to study the dose optimization.
- Full sterility (100%) was recorded in the dose rate 250 and 300 Gy of both male and female pupae. While the sterility was (99.1 and 100 %) of irradiated males and females moths, respectively with the dose rate of 225 Gy while the sterility was reach to (100 %) of F_1 males.
- The percentages of transferred sperm (spermatophore) by irradiated males with 250 and 300 Gy was lower than 225 and 200 Gy. Also, the percentages of spermatophore were increased clearly among the F_1 males as compared to the P_1 at the same doses of 150, 175, 200 and 225 Gy**.**
- Regarding the mating competitiveness value (C.V.) the results indicated that this value was not affected much, either by increasing the dose from 200 to 225Gy or by increasing the ratio from 1: 1 to 1: 3.
- The doses of 200 and 225Gy were fully competitive against untreated males in mating with normal females**.**

Next 18 month plan:

- Studying the dose optimization for *T. absoluta*.

Participants: Ramesh Hire and Ashok Hadapad (India)

5 year plan:

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

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*.

Results from the previous 18 months as planned in RCM2

- Two doses of gamma radiation (150 and 200 Gy) were tested on pupae of *T. absoluta*.
- No significant difference in adult emergence and longevity in both doses as compared to non-irradiated pupae.
- Study on effect of radiation on other reproductive parameters is in progress.

Next 18 months plan

- Complete dose optimization of radiation dose for *T. absoluta*.
- Fitness evaluation of partially sterile males of *T. absoluta.*
- Effect of radiation on reproductive parameters will be completed.

Participants (Muhammad Zahid, Usman Khalique, Noor Fatima, Syed Jawed Ahmad Shah (Pakistan)

5 year plan:

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

Results of previous 18 months:

- No experiments were initiated.

Next 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).

18 months plan

- The objective completed. No further plan.

18 months plan (RCM2 Argentina)

- The objective completed. No further plan.

Next 18 months plan (RCM3 Virtual)

- 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).

18 months plan:

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

Results of the last 18 months as planned during RCM2

- *Optimum conditioning temperature of adults to be irradiated. The mating time and longevity of insects exposed to 2 and 5°C were similar to that of the control individuals at 24° C. On the contrary, insects exposed to 0°C had a shorter mating time and lived shorter than those of the other treatments.

- **Next 18 months plan**

If conditions with respect to covid19 improve in Argentina, we propose:

*To determine the most suitable dose of gamma radiation applied to *T. absoluta* adults.

*To identify the irradiated adults through: 1) the sperm morphology and 2) the genetic polymorphism (RAPD-PCR).

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)

5 year plan:

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

18 month plan:

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

Results of the 18 months

Laboratory evaluation of biological controllers *Trichogramma nerudai, Chrysoperla defreitasi, Goniozus legneri:*

- The percentage of parasitism was evaluated in the laboratory to select the best results, the biological controllers with the best results, will be evaluated in field cages together with sterile moths.

Next 18 month plan:

- *Tupiocoris cucurbitaceus* will be evaluated as a biological controller.

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

5 year plan:

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

18 month plan:

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

Results of the past 18 months:

- The sub-sterilizing dose levels 100, 125 and 150 Gy were used for irradiated males to combine the F1 progeny with T. evanescens against T. absoluta and S. littoralis. The percentages of parasitism of T. evanescens on T. absoluta was higher than S. littoralis, moreover, the parasitism was slightly reduced by irradiation treatments of both species.
- The combination treatment of F1 larvae resulted from irradiated males with sub sterilizing doses as a promising method for T. absoluta and S. littoralis management. Newly hatched larvae were treated with Bt by feeding on tomato leaves treated with the 200 ppm concentration.
- Bacillus thuringiensis is more effective on *S. littoralis* than *Tuta absoluta* and the mortality Percentages were increased gradually by increasing the dose levels.

Next 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 and Ashok Hadapad (India)

5 year plan:

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

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.*

Results from the past 18 months as planned in RCM2

- Parasitization efficiency of five *Trichogramma* sp. and *Trichogrammatoidea bactrae* against *T. absoluta* eggs was assessed.
- *T. pretiosum* parasitized higher number of *Tuta* eggs (58.4%) followed by *Trichogrammatoidea bactrae* (49.2 %) and *T. chilonis* (36.7%).
- The factitious host rice moth was the most preferred host as compared to *S. litura* and *T. absoluta* eggs by *Trichogramma* sp.
- The irradiated eggs of *Corcyra* were accepted and parasitized by *T. pretiosum* and *T. chilonis.*

Next 18 months plan:

- Testing of *T. pretiosum, Trichogrammatoidea bactrae* and *T. chilonis* parasitism efficiency against Tuta control in tomato polyhouse/field conditions.

Participants: Muhammad Zahid, Usman Khalique, Noor Fatima & Syed Jawad Ahmad Shah (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.

Next 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, *Trichrogramma chilonis* (Ishii).
- Low temperature effect on pupae of egg parasitoid, *Trichrogramma 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: Bethylidae) in choice and no choice test under laboratory conditions.

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.

Results of the last 18 months as planned during RCM2

- *The consumption of two types of *T. absoluta* eggs (eggs from untreated parents or from parents with the male irradiated) by small nymphs (2^{nd}-3th instar) of the predator *Tupiocoris cucurbitaceus* was established. Nymphs preyed similar quantities of eggs regardless their origin with a mean consumption of about 43 eggs/nymph.

  *Development and the fecundity of *T. cucurbitaceus* fed on *Tuta absoluta* eggs from parents with the male irradiated. The life history traits of the predator *T. cucurbitaceus* through the lifetable technique on three different diets (*T. absoluta* eggs from irradiated parental males and untreated parents, and *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) eggs (control) were analysed. The nymphal development of *T. cucurbitaceus* was reached in 13 days with a similar survival for all diets (58–65%). Mirid female longevity and oviposition period were statistically similar when the diets consisted of *T. absoluta* eggs from untreated parents and *S. cerealella* eggs, although the number of filial nymphs was larger with the second diet. Moreover, mirid females feeding on *T. absoluta* eggs from untreated parents lived longer and laid double number of nymphs than when fed on *T. absoluta* eggs from irradiated parental males. The highest intrinsic rate of increase (rm) was observed for the *S. cerealella* eggs diet, while differences were not observed between the two types of *T. absoluta*. Therefore, the predator was able to reach adult stage and lay offspring feeding on both types of *T. absoluta* eggs, despite some negative effects of these diets.

Final 18 months plan

*To evaluate the development and fecundity of larval parasitoids (*Goniozus legneri* (Hymenoptera: Bethylidae) and *Pseudapanteles dignus* (Hymenoptera: Braconidae)) on lavae of *T. absoluta* coming from eggs laid by females mated with irradiated males.

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*.

18 month plan:

- No plan for the next 18 months.

Results of the first 18 months:

- No experiments were planned.

Next 18 month plan:

- Assess trap sensitivity under field cages, wild and sterile moths.
- Evaluate natural enemies in field cages in conjunction with the effect of SIT.

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

5 year plan:

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

18 month plan:

- 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.

Results of the first 18 months:

- No experiments were planned.

Next 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 and Ashok Hadapad (India)

5 year plan:

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

Results of the 18 months:

- No experiments were planned.

18 month plan:

- Effect on integration of sterile *T. absoluta* males with selected *Trichogramma* sp. under greenhouse conditions.

Participants (Muhammad Zahid, Usman Khalique, Noor Fatima & Syed Jawad Ahmad Shah (Pakistan)

5 year plan:

- **Results of the Last 18 months**
- *Trichogramma* pupae preservation point of view to use radiation upto 5.0 Gy for maximum adult emergence (83.0%) & longevity (7.33 days).
- Low storage temperature (2°C) is very effective for maximum *Trichogramma* adult emergence (84.67%) & longevity (7.33 days) for 3 days stored pupae.
- The tomato plot (48 m^2) and okra plot (40 m^2) treated with 1500 *Trichogramma* pupae was less infested by fruit worm as compared to untreated plot.
- Maximum population of fruit worm moths were recorded in April i.e., 20.50/ trap followed by March (6.75), September (4.50), July (3.88), May (2.50), November (2.00), June (1.75), Oct. (1.25), August (0.50) & no moth was caught in December, January and February (2019).
- In high tunnel okra plot (24 m^2) treated @ 1500-2000 *Trichogramma* was found less affected by borers as compared to check from July-Nov. (2020).
- Maximum parasitism of *Sitotroga* eggs were recorded upto (33-43%) at a distance of 2-4 feet by *Trichogramma* as compared to 6-10 ft. (7-22%) and also maximum adults of *Trichogramma* were emerged upto 80-93% in tomato pots at glasshouse.
- Low-cost artificial diet-A (chickpea flour base) along with other essential diets ingredients was found very effective to get good quality culture of tomato fruit worm as compared to artificial diet-B (maize flour base) and control diet-C (natural chickpea).
- **Bio-control Training Workshop Conducted during (2019-2020).**
- One day training workshop on "Bio-control of Insect pests of vegetables, field crops and fruit orchards" on April 19, 2019 held at Naguman Farm centre, Peshawar, Pakistan.

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.

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.

Results of the 18 months

The effect of releasing fully Sterile *H. armigera* and integration with *T. brassicae* on tomato fruit infestation in greenhouse was evaluated in following ratios and combinations as bellow:

Experiment 1 and 2:

Section 1: 0 Parasitoid: 0 Sterile male pupae: 1 Normal male pupae: 1 Normal female pupae

Section 2: 0 Parasitoid: 5/10 Sterile male pupae: 1 Normal male pupae: 1 Normal female pupae

Section 3: 132 (number of eggs) Parasitoid: 2.5/5 Sterile male pupae: 1 Normal male pupae: 1 Normal female pupae

- The percentage of infested tomato fruits was 34.47% and 7.85% (56.64% and 88.50% reduction compared to control) when 5 and 10 ratios of irradiated males to normal males and females were released. This value was 43.03% and 14.34% respectively (45.88% and 79.00% reduction compared to control) when 2.5 and 5 ratios of sterile males to normal males and females were released integrated with 132 eggs of *T. brassicae.*

Experiment 3:

Section 1: 265 Parasitoid: 0 Sterile male pupae: 1 Normal male pupae: 1 Normal female pupae

Section 2: 265 Parasitoid: 10 Sterile male pupae: 1 Normal male pupae: 1 Normal female pupae

Section 3: 132 (number of eggs) Parasitoid: 10 Sterile male pupae: 1 Normal male pupae: 1 Normal female pupae

The percentage of infested tomato fruits was 5.4% and 6.10% when a ratio of 10 sterile males to 1 normal males and 1normal females were released integrating 265 and 132 eggs of parasitoid, respectively (compared to 56.90% in control when only normal males and females integrating 265 eggs of parasitoid were released).

Next 18 months plan (RCM3 Virtual):

- To study the effect of F1 generation of treated *H. armigera* on greenhouse crops.
- To determine the effect of integration of *H. armisgera* F1 generation with *Trichogramma* sp. on greenhouse crop.

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*).

18 months plan:

- No plans for the next 18 months

Results of the last18 months as planned during RCM2

- There were no plans for this period.

Final 18 months plan

*To analyse *T. absoluta* population suppression by irradiated adults and their progeny combined with the use of the mirid predator *T. cucurbitaceus* under greenhouse conditions.

2.5. Sex Separation

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

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

5 year plan:

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

18 month plan:

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

Results of the past 18 months:

- Fifth instar larvae were fed with 100, 200, 300, 400, 500 mM concentrations of ethyl methane sulphonate (MES) for 24h. The treated larvae were kept until pupation.
- The emerged adult were sexed and 3 mating categories of each treatment were conducted, treated males X untreated females; treated females X treated male and treated males X treated females. The offspring of each line at each treatment are screening for selectable markers.

Next 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.).

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*.

Results of the last 18 months as planned during RCM2

*Analysis of parthenogenesis in Mar del Plata population of *T. absoluta*. The number of eggs laid by mated females was statistically higher than that observed for unmated females, with a fertility rate of 0.72. The unmated females laid only unfertilized eggs. The longevity was statistically different between unmated and mated females (16.23 vs 12.37 days). Therefore, no evidence of parthenogenesis was obtained in *T. absoluta* wild population from Mar del Plata.

*Detection of reproductive symbionts in four Argentinian populations of *T. absoluta* using 16S rRNA and *gltA* marker and genetic characterization of *Wolbachia* strain/strains by molecular tools (MLST (5 genes) and *wsp)*. Positive results were obtained for *Wolbachia*. Multilocus sequence typing (MLST) and *wsp* sequence analysis determined the presence of a unique *Wolbachia* registered in all individuals analyzed and characterized by the sequence type 41 and allele 10 (*wsp)*. Phylogenetic analysis performed with concatenated sequences of MLST genes clustered *Wolbachia* in Supergroup B. *Ricketssia sp*., *Spiroplasma sp, Cardinium sp.,* and *Arsenophonus sp. W*ere not detected in Argentinian populations.

Final 18 months plan

*To evaluate parthenogenesis in at least one population of *T. absoluta* from another region of Argentina.

*To continue with *Wolbachia* characterization through PCR and sequencing of other loci (groEL, gltA, dnaA, aspC, atp, sucH and pdhB).

Participants: Ramesh Hire and Ashok Hadapad (India)

5 year plan:

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

18 month plan:

Continue to screen wild and laboratory Tuta populations for Wolbachia infections. **Results from the past 18 months as planned in RCM2**

- *Wolbachia* presence in wild and mass-reared *Tuta* samples was assessed using *Wolbachia* specific *16S rRNA* analysis and further characterized by using Multi Locus Sequence Typing (MLST) and *wsp* approaches.
- Around 40-90% *Wolbachia* prevalence was observed in 11 *Tuta* populations.

- Based on MLST and wsp sequence analysis, *Tuta* samples infected with two *Wolbachia* strains (ST41 and ST367).
- Phylogenetic analysis showed that *Wolbachia* strains belong to supergroup A & B.

Next 18 months plan

- Testing of possible IIT event in mass reared *Wolbachia* infected and non-*Wolbachia Tuta* cultures

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 on rearing and quality control protocols for Lepidoptera planned in conjunction with the third RCM was cancelled due to travel restrictions that have been faced during the Covid-19 pandemic.

5. FOURTH RCM LOCATION

The fourth RCM is planned to take place in Sydney, Australia from 8–12 November 2022. A Doodle poll will be sent out to the participants to confirm their availability.

6. LOGICAL FRAMEWORK

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

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

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
	Recommendations for future work	RCM report	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
Activities: 1. Submit CRP proposal. 2. Announce project to MS and amongst established entomologists, biocontrol and pest control specialists and commercial glasshouse growers 3. Organize first RCM to plan, coordinate and review research activities 4. Carry out R&D. 5. Second RCM to analyse data and draft technical protocols as required 6. Hold workshop on "Insect mass rearing for pests of confined cropping systems (*D. suzukii*) and irradiation protocols", in conjunction with second RCM. 7. Continue R&D. 8. Review the CRP after its third year.			Project is approved

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

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ANNEX 1 (LIST OF PARTICIPANTS)

2nd 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)

Third Research Coordination Meeting on Integration of the SIT with Biocontrol for Greenhouse Insect Pest Management

Seibersdorf, Austria (Virtual)

21 to 25 June 2021

Monday, 21 June 2021

SESSION 1

12:00	-	12:15	**Carlos Caceres**: Welcome and introduction

SESSION 2 *D. SUZUKII* PRESENTATIONS

12:15	-	12:45	**Lloyd Stringer:** Estimating *Drosophila suzukii* population density from traps and predicting management tool use.
12:45	-	13:15	**Gustavo Taret:** Development of the sterile insect technique for *Drosophila suzukii*
13:15	-	13:45	**Robin Guilhot:** Comparison of Gamma and X-Ray Irradiation Effects on Sterility and Quality of Spotted Wing Drosophila for Sterile Insect Technique application
13:45	-	14:15	**Alexandra Kruger:** Release of different densities of *Trichopria anastrephae* aiming to control *Drosophila suzukii* in greenhouse.
14:15	-	14:45	**Simone Fellous:** SuperSterile: or how to produce super *Drosophila suzukii* males for sterile releases.
14:45	-	15:00	***Break***
15:00	-	15:30	**Anna Malacrida:** Unravelling *Drosophila suzukii* reproductive dynamics through the seasons in a north Italian region
15:30	-	16:00	**Victor Manuel Gutierrez Palomares:** Pathogenicity and Virulence of *Isaria javanica, Metarhizium anisopliae and Beauveria bassiana strains* for the Control of *Drosophila suzukii* (Matsumura)
16:00	-	16:30	**Pablo Montoya:** Sterility induction, adult emergence and flyers tests for *Drosophila suzukii*

Tuesday, 22 June 2021

SESSION 3 LEPIDOPTERA PRESENTATIONS

12:00	-	12:30	**Silvia Noemi Lopez:** Integration of SIT and biocontrol strategies for the control of the tomato moth *Tuta absoluta* (Lepidoptera: Gelechiidae)
12:30	-	13:00	**Carolina Yáñez Briceño:** Combining Inherited Sterility and *Trichogramma* Egg Parasitoid to Suppress *Tuta absoluta* Population in Tomato Greenhouses.

13:00	-	13:30	**Waheed Ahmed Abdelhamid Sayed:** Improving the Control of *Spodoptera littoralis and Tuta absoluta* using Sterile Insect Technique in Combination with Certain Bio-Control Agents
13:30	-	14:00	**Ramesh Hire:** Identification of potential egg parasitoids and characterisation of *Wolbachia* associated with *Tuta absoluta*
14:00	-	14:15	**Break**
14:15		14:45	**Shiva Osouli:** Evaluation of Integrating SIT and Biological Control against *Helicoverpa armigera* in Greenhouses of Iran
14:45	-	15:15	**Muhammad Zahid:** Environment Friendly management of fruit worm, Helicoverpa armigera (Hub.) through egg parasitoid, *Trichogramma chilonis* (Ishii) coupled with Sterile Insect Technique (SIT) in tomato and okra crops at high tunnel/ field conditions.
15:15	-	15:45	**General discussion**

Wednesday, 23

12:00 SESSION 4 GENERAL DISCUSSION

Selection of working group Chairs and Rapporteurs:

1. D. suzukiiAlexandra Kruger
2. *Spodoptera/Helicoverpa* and *Tuta absoluta Ramesh*

Working Group Discussions, planning and coordinating work programmes

Thursday, 24 June 2021

12:00 SESSION 5 WORKING GROUP DISCUSSIONS (CONTINUED)

Working Group Discussions, planning and coordinating work programmes (continued)

Drafting working group reports and drafting RCM report

12:00 Friday, 25 June 2021

SESSION 6 COMPILING RCM REPORT

Finalizing the RCM report.

General discussion
Announcement of the fourth RCM place and date:
Sydney Australia – 8–12 November 2022 in conjunction with the 11 International Symposium on Fruit Flies of Economic Importance.
Closing

ANNEX 3 (WORKING GROUPS)

Spodoptera/Helicoverpa/Tuta	***Drosophila suzukii***
Carolina Yanes	Simon Fellous
Susana Izquierdo	
Shiva Osouli	Anna Malacrida
Muhammad Zahid	Lloyd Stringer
Ramesh Hire	Robin Guilhot
Cynthia Cagnotti	Gustavo Taret
	Alexandra Krüger
	Victor Gutierrez
	Simone Puppato
	Pablo Montoya

ANNEX 4 (ABSTRACTS OF PRESENTATIONS)

COMPARISON OF GAMMA AND X-RAY IRRADIATION EFFECTS ON STERILITY AND QUALITY OF SPOTTED WING DROSOPHILA FOR STERILE INSECT TECHNIQUE APPLICATION

AUTHOR (S): Robin Guilhot, Keke Gembinsky, Yeudiel Gomez Simuta, Carlos Cáceres

ORGANIZATION: FAO-IAEA – Insect Pest Control Laboratory

SHORT SUMMARY OF PAPER

Abstract:

Recent research activities at the Insect Pest Control Laboratory (Joint Division FAO/IAEA Programme, of Nuclear Techniques in Food and Agriculture) have led to several key achievements that are crucial for the future application of the SIT for the management of *Drosophila suzukii* (Spotted Wing Drosophila, SWD). Among them, suitable male sterilization doses for suppression and eradication strategies were defined using Gamma irradiation. We evaluated – and confirmed – the relevance of using such doses to induce male sterility using instead X-Ray technology, a cheap, safe and accessible alternative to Gamma's. In addition, we compared the effects of Gamma and X irradiation on several quality parameters of SWD (emergence rate, flight ability, longevity, mating behaviour) and seized this opportunity to calibrate QC protocols adapted to this fly species. Ongoing and future projects, that are focused on male sexual behaviour and larval/adult diet, will be briefly described.

A RELEASE OF DIFFERENT DENSITIES OF TRICHOPRIA ANASTREPHAE AIMING TO CONTROL DROSOPHILA SUZUKII IN GREENHOUSE

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:

Biological control has been presented as a potential alternative to the use of insecticides in the management of Drosophila suzukii (Diptera: Drosophilidae), and this technique can also be used associated with other management practices, such as Sterile Insect Technique. Among the most promising biological control agents are the pupal parasitoids, such as Trichopria anastrephae (Hymenoptera: Diapriidae), a species that has already been reported in Brazil and shows potential for parasitism of D. suzukii in laboratory. In the past 18 months we aimed to identify how the release density of T. anastrephae decreases D. suzukii population in a greenhouse. Briefly, we set five walk-in-cages (400 x 300 x 250 cm - length x width x heigth) inside a greenhouse. Inside each cage a table was set with 18 potted strawberry plants, containing at least three fruits per plant. In each cage 60 D. suzukii couples were released, to guarantee infestation. Five days later, T. anastrephae was released in different densities, according to treatment: T1 (control) - 0 parasitoid, T2 - 60 parasitoids, T3 -120 parasitoids, T4 - 240 parasitoids and T5 - 360 parasitoids, in a sex ratio of 0.7. From each treatment, twenty strawberries were collected every five days after parasitoid release for fifteen days, and flies and parasitoids emerged were counted. This experiment setting was repeated 4 times. Our results showed a decrease in number of flies emerged per fruit as the number of parasitoids released increased (T1= 2.32±0.03, T2= 1.83±0.02, T3= 1.15±0.02, T4= 0.94±0.02 and T5= 0.78±0.02 flies/fruit). The number of flies emerged per fruit decreased as time passed after parasitoid release (1st fruit collection= 1.91±0.01, 2nd fruit collection= 1.36±0.01 and 3rd fruit collection= 0.95±0.01 flies/fruit). On the other hand, the number of parasitoids emerged per fruit increased as the number of parasitoids released also increased (T1= 0.00±0.00, T2= 0.12±0.003, T3= 0.16±0.003, T4= 0.25±0.005 and T5= 0.38±0.009 parasitoids/fruit). The number of parasitoids also decreased according to the order of fruit collection (1st= 0.30±0.004, 2nd= 0.15±0.002 and 3rd= 0.09±0.002). Our results showed that releasing pupal parasitoids can decrease D. suzukii population up to 66.38% in a greenhouse.

UNRAVELLING *DROSOPHILA SUZUKII* REPRODUCTIVE DYNAMICS THROUGH THE SEASONS IN A NORTH ITALIAN REGION.

AUTHOR (S):Simone Puppato[1] , Davide Carraretto[2], Ludvik Gomulski[2], Giuliano Gasperi[2], Carlos Caceres[3], Alberto Grassi[1], Claudio Ioriatti[1], Anna Malacrida[2]

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[3]International Atomic Energy Agency, Vienna International Centre, PO Box 100 1400 Vienna, Austria

SHORT SUMMARY OF PAPER

Abstract:

The rapid and extensive establishment of *D. suzukii* outside its indigenous regions has been facilitated by its tolerance to a broad range of climatic conditions, and by its high reproductive potential. This fly displays several seasonal adaptive traits which allow it to overwinter and therefore to establish and expand in temperate regions. Overwintering of adult males and females and early season reproductive behaviour play important roles in the seasonal build up of the populations. This has severe implications for fruit cultivation. On this background, our attention has been centred on the dynamics of the reproductive biology of *D. suzukii* populations across the seasons in an area of Trentino which is 1) rich in greenhouses for fruit cultivation and 2) maintains established population of *D. suzukii* that are able to overwinter. In this area the females are reproductively active from early spring until late autumn, and the most stringent bottleneck for *D. suzukii* populations is from January to March. Overwintered *D. suzukii* females are thought to store sperm from autumn matings to counteract the winter bottleneck that may result in a scarcity of mature males in early spring. This strategy would allow them to resume oviposition when they exit reproductive diapause in the early spring, without needing to mate again. Given this background, an important unexplored aspect of *D. suzukii* reproductive behaviour is the assessment of the number of times that a female mates in the wild across the seasons. This influences the effective population size and may constitute a critical factor in determining the success of control methods. Using molecular markers, we are analysing the presence and the extent of polyandry across seasons and we are assessing the use of sperm by females. These aspects of *D. suzukii* mating behaviour may locally be a constraint to the application of the environmental friendly control methods such as the Sterile Insect Technique (SIT).

PATHOGENICITY AND VIRULENCE OF *ISARIA JAVANICA*, *METARHIZIUM ANISOPLIAE* AND *BEAUVERIA BASSIANA* STRAINS FOR THE CONTROL OF *DROSOPHILA SUZUKII* (MATSUMURA)

AUTHOR (S): Victor Manuel Gutierrez-Palomares, Luis Paulino-Alonso, Jorge Zambrano Gutierrez,

Raquel Alatorre-Rosas

ORGANIZATION: SENASICA

SHORT SUMMARY OF PAPER

Abstract:

The pathogenicity and virulence of 3 strains of entomopathogenic fungi *Isaria javanica* (CHE-CNRCB 307), *Metarhizium anisopliae* (CHE-CNRCB 224) and *Beauveria bassiana* (CHE-CNRCB 168) plus a control of 1ml of sterile Tween® 80 at the 0.03% on adults of the Spotted Wing Vinegar Fly *Drosophila suzukii* were evaluated. Lethal Concentration 50 (LC_{50}) of entomopathogenic fungi was determined at 5 concentrations ($1X10^4$, $1X10^5$, $1X10^6$, $1X10^7$ and $1X10^8$ conidia/ml^{-1}) and the Lethal Time (LT_{50}) at a dose of $1X10^8$ conidia/ml^{-1}.

The evaluated strains showed different degrees of pathogenicity and virulence on the adults of *D. suzukii*. The strain of *I. javanica* (CHE-CNRCB 307) was the one that registered the largest significant differences with an LC_{50} of $1.6x10^4$ adult conidia/ml^{-1} and a LT_{50} of 4,579 days. The strains of *M. anisopliae* (CHE-CNRCB 224) and *B. bassiana* (CHE-CNRCB 168) were not significantly different when compared with each other with an LC_{50} of $3.1x10^5$ and $1.4x10^6$ adult conidia/ml^{-1} and LT_{50} of 18.16 and 15.3 days respectively.

The results of the present study provide support for the control of *D. suzukii* with entomopathogenic fungi.

STERILITY INDUCTION, ADULT EMERGENCE AND FLYERS TESTS FOR *DROSOPHILA SUZUKII*

AUTHOR (S): Emilio Hernández, Marysol Aceituno-Medina, Rubén Hernández, Pablo Montoya

ORGANIZATION: Programa Moscas de la Fruta SENASICA-SADER, Camino a los Cacaotales S/N, PC 30860, Metapa de Domínguez, Chiapas, Mexico

SHORT SUMMARY OF PAPER

Abstract:

A new colony of *Drosophila suzukii* (Matsamura) was established at the Moscafrut facilities using a carrot larvae diet, which consist in a mix of 7.3% carrot fibre, 8.3% corn flour, 6.3% Torula yeast, 9% table sugar, 0.33% sodium benzoate, 0.2% Nipagin TM and 0.4% citric acid. To determine the sterility induction under laboratory populations, we first established the optimal age at which the pupae should be irradiated at 200 Gy, quantifying the percentage of eyes colour of pharate adults from 4-day-old pupae. According to the results, the individuals of *D. suzukii* reared with carrot fibre diet and Torula yeast showed seven well-defined ages, 0, -6, -12, -18, -24, -36, and 48 h before the adult emergence, finding that only 66% of the pupae were at the optimum age to be irradiated (-12 h). We also evaluated the flight ability using different containers, including the 9x9 cm black PVC tube. The emergence of adults corresponded to 65.29 ± 3.64, 65.08 ± 3.06, and 84.17 ± 3.06% using a tube of 9x9, 9x5, and 4x8 cm (height x diameter), respectively. The percentage of adult flyers obtained were 59.57 ± 3.39, 59.63 ± 2.56, and 78.33 ± 3.11% using a 9x9, 9x5, and 4x8 cm tube (height x diameter), respectively. For the sterility induction test, the ratios of sterile male: fertile male: fertile female under evaluation correspond to 0:10:10, 10:10:10, 50:10:10, and 100: 10: 10 using Plexiglas cages of 30x40x30 cm. This test is still under evaluation.

ESTIMATING *DROSOPHILA SUZUKII* POPULATION DENSITY FROM TRAPS AND PREDICTING MANAGEMENT TOOL USE

AUTHOR (S): Lloyd Stringer

ORGANIZATION: The New Zealand Institute for Plant and Food Research Ltd.

SHORT SUMMARY OF PAPER

Abstract:

The ability to estimate the population density of spotted wing drosophila, SWD, *Drosophila suzukii,* helps to inform when management tools need to be applied. This is especially true for population management tools that act in a density dependent manner. These include tools like the sterile insect technique and insect biological control agents, whereby the density at which they can be released in comparison to the pest density improves management outcomes. This is because their effect follows a probabilistic response, so the more a sterile insect or parasitoid can over flood the target life stage, the greater the probability that they will interact with the target, e.g. mate or parasitise.

I will present updates to a population estimate model for spotted wing drosophila based on catch in traps, and an approach to estimate the effects of combining the sterile insect technique with a biological control agent to achieve low pest prevalence and maintain low to nil-residue fruit production.

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

AUTHOR (S): Carolina Yáñez Briceño Ing. Agr., Susana Izquierdo Carreño Ing. Agr., David Castro da Costa Ing. Agr., George Saour.

ORGANIZATION: Fundación Para el Desarrollo Frutícola (FDF)

SHORT SUMMARY OF PAPER

Abstract:

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is one of the most destructive insect pests of tomato plants worldwide. It has also been reported that the insect feeds on other hostplants from the Solanceae family. *T. absoluta* is controlled by the application of highly dangerous insecticides with wide spectra of action.

The effects of sterile insect technique/inherited sterility technique (SIT/IS) on *T. absoluta* as a possible control tactic had been already proposed. Moreover, it is essential to have suppression alternatives such as combining biological control agents with SIT/IS which have great potential as complementary and environmentally friendly control method.

The FDF was carried out several bioassays on *T. absoluta* and the most important results are the following:

Several artificial diets for *T. absoluta* rearing were tested, which are important to develop new control approaches against *T. absoluta*.

T. absoluta colony under laboratory conditions was maintained where biological parameters such as larval viability, larval duration, pupal recovery, and adult emergence of *T. absoluta* were determined on different artificial diets and tomato leaves.

The required sterilization doses for male and female adults with a new evaluation methodology were established.

Preliminary laboratory experiments were carried out to evaluate the efficiency of *Trichogramma nerudai*, *Crysoperla defreitasi* and *Goniozus legneri* as bio control agents against *T. absoluta*. An initial release of moths in cages under field conditions was performed.

IMPROVING THE CONTROL OF *SPODOPTERA LITTORALIS* AND *TUTA ABSOLUTA* USING STERILE INSECT TECHNIQUE IN COMBINATION WITH CERTAIN BIO-CONTROL AGENTS

AUTHOR (S): Sayed, W.A.A.[1]; Sileem Th.M.[1]; Hassan R.S.[1]; Elhelaly, A.[2] and Hassan, G.[3]

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SHORT SUMMARY OF PAPER

Abstract:

The efficacy of sterile insect technique (SIT) and different bio control agents against *Spodoptera littoralis* and *Tuta absoluta* for concurrent use was assessed and it does indeed have a multivariate optimization perspective, taking into account the mass rearing of sterile insect, dose optimization of gamma ray and interaction of the different control methods.

The lowest cost diets based on gelatin and starch ingredient were evaluated for rearing *S. littoralis* larvae as substitute of expensive gelling agent (agar agar). Although, the larvae exhibited developmental plasticity and were capable of development successfully, the deleterious effect of certain biological and physiological properties was observed. Rearing *S. littoralis* larvae on castor leaves-based diet and agar-based diet were exhibited high quality sterile insect for SIT application. While, the modified diet was more effective for *Spli*MNPV production as compared with the standard diets. On the other hand, *T. absoluta* was reared and maintained in the laboratory conditions for many generations on tomato plant. Furthermore, different artificial diets were tested for rearing *T. absoluta*, however, the pupal and adult production were insufficient to recommend one of them as a successful rearing diet for SIT program.

Full-grown male and female pupae of *S. littoralis* and *T. absoluta* were irradiated with the dose levels 100, 150, 200, 225, 250, 300 and 350 Gy to determine the optimal dose that could be applied. The results indicated that 225 Gy induced (98 and 100 %) sterility in the parent (P_1) and first generation (F_1) male, respectively, while 100% sterility was recorded in the P_1 female moth. The mating competitiveness values were slightly affected by the dose levels from 100 to 225 Gy. Furthermore, the dose levels of 250 and 300 Gy exhibited 100% sterility of *T. absoluta* when irradiated as full grown pupae.

Integration of SIT and bio-control methods revealed that the *Bacillus thuringiensis* was effective against both *S. littoralis* and *T. absoluta*. While, the egg parasitoid, *Trichogramma evanescens* was more effective for *T. absoluta* than *S. littoralis*, the parasitized egg percentages were (97.1 and 75 %), respectively. Moreover, the parasitism was insignificantly influenced by the irradiation treatments.

IDENTIFICATION OF POTENTIAL EGG PARASITOIDS AND CHARACTERISATION OF *WOLBACHIA* ASSOCIATED WITH *TUTA ABSOLUTA*

AUTHOR (S): Ramesh S. Hire and Ashok B. Hadapad

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 a major pest of solanaceous crops and distributed in Europe, North African Mediterranean basin and many Asian countries. This invasive insect pest was first observed in India during 2014-15 and causes nearly 90% damage to tomato crop. During this period of IAEA-CRP, we studied the *Tuta* prevalence in Northern and Eastern part of India, mass rearing optimization of *Tuta* and parasitization efficiency of *Trichogramma* species on *Tuta*, *Spodoptera* and *Corcyra cephalonica* eggs under laboratory conditions. We also assessed the *Wolbachia* prevalence in wild and mass-reared *Tuta* populations.

T. absoluta was more prevalent in Southern and Western states of India as compared to Northern and Eastern states. Mass rearing of tomato leaf miner culture was optimised on tomato seedlings in insect rearing and cloth cages resulting in continuous supply of different stages for experiments. Parasitization efficiency of five *Trichogramma* sp. and *Trichogrammatoidea bactrae* against *T. absoluta* eggs was assessed. Among the *Trichogramma* sp., *T. pretiosum* parasitized higher number of *Tuta* eggs (58.4%) followed by *Trichogrammatoidea bactrae* (49.2 %) and *T. chilonis* (36.7%). The factitious host rice moth was the most preferred host as compared to *S. litura* and *T. absoluta* eggs by *Trichogramma* sp. *Wolbachia* presence in wild and mass-reared *Tuta* samples was assessed using *Wolbachia* specific *16S rRNA* analysis and further characterized by using Multi Locus Sequence Typing (MLST) and *wsp* approaches. Around 40-90% *Wolbachia* prevalence was observed in 11 *Tuta* populations carrying two *Wolbachia* strains (ST41 and ST367) those belong to supergroup A & B. These results will be further evaluated under pilot field condition in order to integrate with SIT for efficient management of *T. absoluta*.

EVALUATION OF INTEGRATING SIT AND BIOLOGICAL CONTROL AGAINST *HELICOVERPA ARMIGERA* IN GREENHOUSES OF IRAN

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SHORT SUMMARY OF PAPER

Abstract:

Evaluation of Integrating SIT and Biological Control against *Helicoverpa armigera* in Greenhouses of Iran

Helicoverpa armigera Hübner (Lepidoptera: Nuctuidae), is a world-known polyphagous pest which it's outbreak in some years has resulted in up to 80 % damage in several crops in Iran. *H. armigera* larvae prefer to feed on reproductive parts of over 180 cultivated hosts. High fecundity, short generation period, ability to survive in various habitats, polyphagy, easy adaptation to environmental changes, and facultative diapause of *H. armigera* make it a key and serious pest for various crops. Tomato, as one of the major host plants of this pest, is the second most important greenhouse product in Iran after cucumber. The sterile insect technique (SIT) or/and inherited sterility (IS) offer great potential as effective and environmentally friendlier control tactics against this pest. For this purpose, the effect of releasing two ratios of sterile males (irradiated with 350 Gy) of *H. armigera* alone and also integrated with *Trichogramma brassicae* on tomato crop infestation in research greenhouse were evaluated from April 2019 to February 2020.The percentage of infested tomato fruits was 34.47% and 7.85% (56.64% and 88.50% reduction compared to control) when 5 and 10 ratios of irradiated males to normal males and females were released. This value was 43.03% and 14.34% respectively (45.88% and 79.00% reduction compared to control) when 2.5 and 5 ratios of sterile males to normal males and females were released integrated with 132 eggs of *T. brassicae.* These results indicated that the number of fruits with larval infestation increased when the ratio of sterile males decreased by half even though was integrated with half amount of parasitoid eggs (compared to releasing only sterile males in our experiments). The percentage of pupal mortality was 2.5-7% in normal pupae and 6-8% in irradiated ones. The effect of releasing sterile and partially sterile males (irradiated with 350 Gy and 200 Gy, respectively) of *H. armigera* integrated with *Trichogramma brassicae* on tomato crop infestation in research greenhouse were evaluated from February 2020 to February 2021. The percentage of infested tomato fruits was 5.4% and 6.10% when a ratio of 10 sterile males to 1 normal males and 1normal females were released integrating 265 and 132 eggs of parasitoid, respectively (compared to 56.90% in control when only normal males and females integrating 265 eggs of parasitoid were released). This value was 53.96% and 57.62% respectively when a 10 ratio of partially sterile males to 1 normal males and 1 normal females were released integrating 265 and 132 eggs of parasitoid, respectively (compared to 86.84% in control when only normal male and female were released).

ENVIRONMENT FRIENDLY MANAGEMENT OF FRUIT WORM, *HELICOVERPA ARMIGERA* (HUB.) THROUGH EGG PARASITOID, *TRICHOGRAMMA CHILONIS* (ISHII) COUPLED WITH STERILE INSECT TECHNIQUE (SIT) IN TOMATO AND OKRA CROPS AT HIGH TUNNEL/ FIELD CONDITIONS (IAEA RES. CONTRACT-21002)

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(SHORT SUMMARY OF PAPER)

Abstract

Egg parasitoid, *Trichogramma chilonis* (Ishii) is a useful bio-control agent for the control of many lepidopterist insect pests. Radiation dose (5.0 Gy) and low storage temperature (2°C) for three days were found suitable for maximum adult emergence (83.0-84.67%) & longevity (7.33 days) of *Trichogramma* pupae (2019). Low radiation and cold storage increases shelf life of *Trichogramma* pupae without maximum detrimental effects on quality of parasitoids during transportation and ensure year around availability in insectary for research/field releases.

In high tunnel tomato and okra plot (48 & 40 m^2) treated with *Trichogramma* wasp (1500 nos. pupae installed card were less infested by fruit worm, *H. armigera* i.e., 0.41 & 0.35/plant followed by 1000 pupae (0.67 & 0.63/plant), 500 pupae (0.85 & 0.77/plant) as compared to control plot i.e., 1.30 & 1.13/plant. Production was also higher in 1500 pupae/plot i.e., 32.88 & 24.2 kgs followed by 1000 pupae (27.46 & 20.50 kgs) and 500 pupae (22.33 & 17.88 kgs.) as compared to control i.e., 17.75 & 13.29 kgs in tomato and okra respectively (2019).

Maximum fruit worm moths were recorded in pheromone traps in the month of April i.e., 20.50/ trap followed by March (6.75), September (4.50), July (3.88), May (2.50), November (2.00), June (1.75), October (1.25), August (0.50) and no moth was found in December, January and February (2019).

Minimum fruit worm, *H. armigera* and spotted bollworm, *Earias vitella* (F.) infestation was recorded i.e. 0.20 & 0.50/okra plant in *Trichogramma* wasps (2000 nos. pupae installed card) treated plot (24 m^2) followed by 1500 pupae card (0.27 & 0.58), 1000 pupae card (0.32 & 0.67) and maximum in control plot (no. pupae installed card) i.e. 0.40 & 0.92/okra plant respectively in high tunnel okra from July to November (2020). Okra plot treated @ 1500-2000 *Trichogramma* was found less affected by borers as compared to control.

Maximum parasitism of Angoumois grain moth, *Sitotroga cerealella* (Oliv.) eggs by *Trichogramma* wasps were recorded upto (33.00-43.00%) at a distance of 2.0-4.0 feet whereas minimum parasitism (7.00-22.00%) at a distance of 6.0-10.0 feet in tomato pots in glasshouse. Maximum *Trichogramma* wasps were emerged upto 80-93% from these parasitized eggs cards when brought into bio-control lab. So, parasitism of *Sitotroga* eggs is inversely proportion to distance covered by the *Trichogramma.*

Fruit worm, *Helicoverpa armig*era (Hub.) is a major pest of tomato and okra in Pakistan. Artificial diets-A (chickpea flour base and artificial diet-B (maize flour base) were tested for the rearing of *H. armigera* compared with control diet-C (natural chickpea leaves, pods) in laboratory (2020). During the biological study of various parameters that the minimum larval duration of fruit worm was recorded upto 12.0 days in artificial diet-A followed by control diet-C (14.3 days) & artificial diet-B (15.9 days). Larval mortality was observed minimum (6.2%) in artificial diet-A followed by artificial diet-B (8.6%) and Control diet-C (15.6%). Pupal duration was observed minimum (9.0 days) in artificial diet-A followed by artificial diet-B (12.6 days) & control diet-C (14.0 days). Maximum pupal recovery was recorded in artificial diet-A i.e., 86% followed by control diet-C (82%) and artificial diet-B (71%). Maximum male moths were recorded in artificial diet-B and control diet-C 56-60% and minimum in artificial diet-A (32%).

Maximum female moths were recorded in artificial diet-A i.e., 68% followed by artificial diet-B & control diet-C (40-40%). Maximum moths were emerged in artificial diet-A i.e., 86% followed by control diet-C (82%) & artificial diet-B (76%). Low cost artificial diet-A (chickpea flour base) along with other essential diets ingredients is very effective to get good quality culture of *H. armigera* in laboratory.

Key words: Bio-control agent, *Trichogramma chilonis* (Ishii), *Helicoverpa armigera* (Hub.), *Earias vitella* (F), artificial diets, insect rearing, pheromone baited trap.

SUPERSTERILE: OR HOW TO PRODUCE SUPER DROSOPHILA SUZUKII MALES FOR STERILE RELEASES

AUTHOR (S): Allan Debelle, Julie Collet, Anne Xuéreb, Joris Froissard, Clelia Oliva and Simon Fellous

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SHORT SUMMARY OF PAPER

Abstract:

The SuperSterile project (2018-2020) aimed at identifying male sexual characters preferred by *Drosophila suzukii* females and develop a genetic line of super males to improve the efficacy of sterile releases. In a female-choice experiment, we compared male wing phenotype – size, color, spots – with mating success. This revealed features females *D. suzukii* use to chose their mates. Using experimental evolution in the laboratory we selected *D. suzukii* lines for improved mating performance. Preliminary screening in lab conditions showed 3 such lines were promising. These 3 lines were tested in mesocosmes that contained natural plants, fruits and wild females. This experiment highlighted the superior mating propensity of one of the lines which was preferred by wild females over control males. This superiority maintained even when males were sterilised with X-rays. This super-line will be used for the field validation of the SIT on *D. suzukii* in France.

INTEGRATION OF SIT AND BIOCONTROL STRATEGIES FOR THE CONTROL OF THE TOMATO MOTH *TUTA* ABSOLUTA (LEPIDOPTERA: GELECHIIDAE)

AUTHOR (S): López S.N., Conte C., Lanzavecchia S. and Cagnotti C.

ORGANIZATION: INTA

SHORT SUMMARY OF PAPER

Abstract:

Tuta absoluta (Lepidoptera: Gelechiidae) is a South American lepidopteran that usually attacks plant species from the Solanaceae family. It is considered a main pest of tomato both in cultivated fields and greenhouses. In 2006, its presence was reported in Spain, and since then it has been spreading throughout Europe and also invading Asia and Africa. The objectives of the present work were: 1- To study the integration of the IS technique with the biological control agents present in our tomato crops areas; 2- to evaluate the capacity of *T. absoluta* populations from Argentina to reproduce parthenogenetically under laboratory conditions and 3- to detect the presence of endosymbionts associated to reproductive abnormalities. The methods and results for each objective are the following:

1-Regarding the first objective, we analysed the consumption of *T. absoluta* eggs by small nymphs (2^{nd}-3^{rd} instar) of the predator *Tupiocoris cucurbitaceus* (Hemiptera: Miridae). Two types of *T. absoluta* eggs, i.e., eggs from untreated parents or from parents with the male irradiated were studied. Seventy eggs of each cross type were transferred to the surface of a tomato leaflet in a Petri dish and a *T. cucurbitaceus* small nymph was introduced into this arena for 24 h. The number of partially or fully consumed eggs was recorded. Nymphs preyed similar quantities of eggs regardless their origin with a mean consumption of about 43 eggs/nymph. We also evaluated the life history traits of the predator *T. cucurbitaceus* through the lifetable technique on three different diets: *T. absoluta* eggs from irradiated parental males and untreated parents, and *Sitotroga cerealella* (Olivier) (Lepidoptera:Gelechiidae) eggs (control). The nymphal development of *T. cucurbitaceus* was reached in 13 days with a similar survival for all diets (58–65%). Mirid female longevity and oviposition period were statistically similar when the diets consisted of *T. absoluta* eggs from untreated parents and *S. cerealella* eggs, although the number of nymphs laid was larger with the second diet. Moreover, mirid females feeding on *T. absoluta* eggs from untreated parents lived longer and laid double number of nymphs than when fed on *T. absoluta* eggs from irradiated parental males. The highest intrinsic rate of increase (rm) was observed for the *S. cerealella* eggs diet, while differences were not observed between the two types of *T. absoluta*. Therefore, the predator was able to reach adult stage and lay offspring feeding on both types of *T. absoluta* eggs, despite some negative effects of these diets.

2- Larvae of *T. absoluta* collected from commercial greenhouses of Mar del Plata, Buenos Aires province were reared on tomato plants in the Insectario de Investigaciones para Lucha Biológica (IILB), IMYZA, INTA (Castelar, Buenos Aires, Argentina). To study the presence of parthenogenetic reproduction, 22 females 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. In both treatments the leaves were changed every 3 days until female death. The number of eggs laid, the proportion of emerged larvae and the longevity of females were recorded. Besides, females and males adults were conserved to evaluate the presence of endosymbionts in molecular studies. The number of eggs laid by mated females was statistically higher than that observed for unmated females, with a fertility rate of 0.72. The unmated

females laid only unfertilized eggs. The longevity was statistically different between unmated and mated females (16.23 vs 12.37 days). Therefore, no evidence of parthenogenesis was obtained in *T. absoluta* wild population from Mar del Plata.

3-*R*eproductive symbionts were assessed in adult individuals of *T. absoluta* from four localities of Argentina by standard PCR. Reactions were conducted using specific primers to detect *Wolbachia sp*., *Ricketssia sp*., *Spiroplasma sp, Cardinium sp.,* and *Arsenophonus sp.* Positive results were obtained for *Wolbachia*. Further multilocus sequence typing (MLST) and *wsp* sequence analysis determined the presence of a unique *Wolbachia* registered in all individuals analyzed and characterized by the sequence type 41 and allele 10 (*wsp)*. Phylogenetic analysis performed with concatenated sequences of MLST genes clustered *Wolbachia* in Supergroup B. Future analysis of possible way of transmission and the effect of infection by *Wolbachia* in *T. absoluta* will bring a new insight in the study of this pest species and potential efficient and sustainable control strategies.

DEVELOPMENT OF THE STERILE INSECT TECHNIQUE FOR *DROSOPHILA SUZUKII*

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SHORT SUMMARY OF PAPER

Abstract:

The development of Sterile Insect Technique for Drosophila suzukii represents for our region the possibility of offering to our berries growers an answer for the control of a pest especially under greenhouses conditions and on the future explore the possibility to be applied on open field.

The work carried out in the last 18 months has been seriously affected by strong operational restrictions (lockdown), implemented in Argentina as a result of the Covid19 pandemic from the beginning of 2020 and still continue. However, it was possible keep lab colony to enable the evaluation of the main parameters proposed.

A refreshing of our colony was possible due introduction of wild flies, collected on the north of our country and each strain were compared trough out five generations, The next quality parameters of the insects were monitored: Pupal weight, Flight ability, and Survival under stress. According to the results obtained, no significant differences were observed between lab strain and wild strain, although a better performance of flight ability was observed on wild flies.

Wild flies were kept on fruit for larvae development while larvae of lab strain were reared on artificial larvae diet.

Regarding pupal separation system, the centrifuge system was adjusted for the separation of pupae. The most appropriate separation age is under study to minimize the impact of mechanical separation on quality.

An egg collection system with gelling material was evaluated, resulting in an average of 2.6 eggs/♀/day throughout 8 oviposition days. The extension of oviposition time to 12 days declined eggs production to 1.86 eggs/♀/day.

A competitiveness assessment was carried out under greenhouse conditions for four consecutive release weeks at the rate of two times per week. A competitiveness ratio of 100: 1 (Sterile: Fertile) was evaluated. The irradiation dose evaluated was 180 Gy. Fried competitiveness index was applied to evaluate the competitiveness, using strawberries and larval diet devices as eggs oviposition place for feamles. The results obtained resulted in an average index of 0.31 (1.16 / 0.16). The environmental conditions during the development of the test offered average temperature values of 16.8 ° C with a maximum of 31.9 ° C and a minimum of 2.5 ° C. Relative humidity ranged 56.5% on average with a maximum of 94.7% and a minimum of 24.7%. At the same time, pre and post irradiation flight ability evaluations were carried out, observing pre-irradiation emergency values of 72.07% (88.7 / 68.25) and a percentage of flyers of 69.31% (86.0 / 50.0). The values obtained after irradiation with a dose of 180 Gy had an emergence of 61.10% (76.0 / 47.0), while percentage of the flyers had an average of 41.11% (49.4 / 30.55), strongly denoting the effect of the irradiation dose applied.

ANNEX 5

DIET RECIPES USED BY THE PARTICIPANTS FOR REARING *D. SUZUKII* AND SOME SPECIES OF LEPIDOPTERA

From RCM3 – Virtual, 21–25 June 2021

D. suzukii:

Insect Pest Control Laboratory, IAEA, Seibersdorf, Austria

Diet 1: Potato

	For 1 Kg
Potato flakes	130 g
Yeast	70 g
Sugar	65 g
Sodium benzoate	3 g
Nipagin	3 g
Water	700 ml
HCl (32% solution)	<2ml (PH must be >2.8)

Diet 2: Wheat bran

	For 5 Kg
Wheat bran	1400 g
Yeast	350 g
Sugar	650 g
Sodium benzoate	21 g
Nipagin	21 g
Water	3 L

Gustavo Taret -Iscamen Mendoza - Argentina

Diet Potato - 1

	For 100 kgX
(ingredients)	(quantities/volumes)
Potato powder	19.34 Kg
Carrot powder	8.29
Sugar	11.05
Torula yeast	4.87
Sodium Benzoate	0.21
Methil paraben	0.97
Water	55.27

Alexandra Krüger Universidade Federal de Pelotas and EMBRAPA, Brazil

	For 1 L
Cornmeal	80 g
Yeast	40 g
Sugar	100 g
Agar	8g
Propionic acid	3 mL
Nipagin (at 10 % -distilled in ethanol)	8mL
Water	1 L

Lepidoptera species

Tuta absoluta: