CRP Title:
" Development of mass rearing for New World (Anastrepha) and Asian (Bactrocera) fruit fly pests in support of sterile insect technique (SIT)

Section/Division: Insect Pest Control / Joint FAO/IAEA Division (NAFA)

Project Officer: Andrew Jessup

Period Covered: 2004 - 2010

Objectives of CRP:

(a) Overall (Agency Project towards which CRP directed):

To develop and improve rearing technology for selected *Anastrepha* and *Bactrocera* fruit flies of economic importance for SIT application.

(b) Specific (CRP):

1. To improve/develop mass-rearing diets used in rearing of selected *Anastrepha* and *Bactrocera* fruit fly pests.

2. To assess the impact of biotic and abiotic factors on production and quality of mass-reared fruit flies.

3. To increase the efficiency and cost effectiveness of mass-rearing fruit flies for SIT.

4. To develop improved handling, process control and automation of mass-reared fruit flies.

Outputs:

(a) Research:

1. New Pest Species Being Colonized

1.1. *Anastrepha zenildae*

- The states of Ceara and Rio Grande do Norte are very important fruit producing states for export and local consumption. In the fruit producing areas of these two states a complex of *Anastrepha* species was identified where *A. zenildae* is the predominant species with over 80% of the standard population. During the CRP, experiments were conducted in order to identify and rear these species under laboratory conditions.
- A colony of *A. zenildae* was established at the lab of EMBRAPA, Agroindustria Tropical. A larval diet based on 18% sugar cane bagasse was found to improve pupa to adult recovery although adult mortality within the first 15 days post-eclosion is still too high.
- The best oviposition device was an agar agar panel over a glass bottle.
- Mating compatibility between this species and *A. fraterculus* was analysed with populations from Ceara, Rio Grande do Norte and Sao Paulo. No viable eggs were produced.

1.2. *Bactrocera pyrifoliae*

- We began the CRP by documenting the host plants of *B. pyrifoliae* in northern Vietnam. Our survey showed that the fly attacks 2 major host plants of economic importance namely peach (*Prunus salicina*) and plum (*Prunus persica*). The survey indicated that *B. pyrifoliae* occurs only in mountainous regions at elevations from 900-1400 m above sea level.

- In an attempt to establish the most suitable temperature and relative humidity for rearing, it was observed that temperature range of 25-37^{0}C in the daytime and 20-22^{0}C by night and RH range of 75-85% was the most efficient for the first 3 generations. Rearing at subsequent generations can be maintained at 22-24^{0}C and RH of 75-85%.
- Several diet types were tested based on carrot, papaya, guava and mango for mass rearing of *B. pyrifoliae* and the most suitable was found to be the carrot diet based on all quality control parameters and especially pupal weight.
- The diet was compared with other locally available raw materials such as corn, soybean, rice and a combination of the three. The results depicted carrot as the most suitable among the 5 diet types. However, a combination of carrot and corn was found to be the most suitable and cost effective for rearing *B. pyrifoliae*.
- The most effective fly number based on low mortality in the cages was 1000 flies per cage
- The male to female ratio that provided the most efficient production in terms of % egg hatch was found to be 2:3 at 400 males to 600 females.
- The currently used adult diet consists of yeast, sugar and water at a ratio of 20:80:1%. In addition we also provide bacteria.

1.3. *Bactrocera invadens*

- Upon discovery in 2003, *B. invadens* was maintained on whole mango fruits. In the course of the CRP, effort was made to move the insect from whole fruit rearing to artificial diet and the adaptation process of the *B. invadens* when moved from whole mango fruit rearing to artificial diet based on wheat-bran, took between three to five generations to reach the plateau of quality control parameters observed for rearing the insect on whole mango fruits.
- In further studies we were able to demonstrate that *B. invadens* could also be successfully reared on diet based carrot-, sugarcane bagasse-, and cassava-based but quality control parameters for the cassava-based diet was significantly lower than what was observed for the other diets.
- Demographic parameters were generated on carrot based diet.
- The production of *B. invadens* on liquid diet was also assessed during the past period of the CRP and results clearly showed that the insect can be successfully reared on liquid diet. Quality control parameters often exceeded those obtained for carrot-based solid diet.
- Five oviposition devices were tested and *B. invadens* readily accepted plastic bottle, funnel, plastic cup and mango dome for egging but to varying degrees. The plastic bottle was found to be the most effective for egg collection and maintaining high fertility.
- Attempts to replace the standard enzymatic yeast hydrolysate with local yeast for adult feeding were not successful. Over the 10-day standard egg collection period, average egg production from the flies fed the standard enzymatic yeast hydrolysate was significantly higher compared with the flies fed on either the Kenyan yeast, Ugandan yeast or Mauritian yeast. Eggs hatch was also significantly affected by the diet type; it was highest in the standard yeast compared to the other diets.
- In molecular studies, 11 microsatellite markers were characterized.
- Analysis of invasion history showed that the Sri Lankan population is a well established population that was well differentiated with high numbers of private allele demonstrating the Asian origin of the pest.
- Two points of invasion into Africa were found to be Kenya and Benin, and the oldest migrations are from Kenya where the pest was first detected while Benin is the secondary spread point.
- All the populations from Africa seem to be well differentiated a situation showing that *B. invadens* could have been well established before its detection in Africa
- DNA barcoding using the CO1 gene showed that *B. invadens* from Kenya, Uganda, and some individual from the Nigeria and Sri Lankan populations clustered together with *B. kandiensis*. *Bactrocera dorsalis* s.s. clustered separately from all *B. invadens* samples. *Bactrocera oleae*, *B. zonata* and *B. cucurbitae* samples generally had the highest values of the genetic distance, an indication that these samples are genetically distanced from the other *Bactrocera* species.

1.4. *Bactrocera xanthodes* and *B. kirki*

- Laboratory colonies of each of these two species based on fresh papaya fruit were established as part of this CRP.
- A liquid larval diet was tested under collaboration with the USDA. Results indicated that these two species could be reared on the liquid diet (brewer's yeast, sodium benzoate, wheat germ oil, ascorbic acid and water). More testing needs to establish its impact on all relevant quality factors.
- Of the four types tested nn egg collection device consisting of a 2.5mL plastic syringe punctured with fine holes and coated on the inside with fruit juice (guava, abiu or banana) was found to collect the most eggs.

1.5. *Ceratitis cosyra*

- Various oviposition stimulants were tested and marula juice was found to be a suitable oviposition stimulant.
- Several artificial larval diets were tested for production of *C. cosyra* and a combination of carrot and mango powder was found to be the most effective for larval development.
- *Ceratitis cosyra* was also found to successfully develop on liquid diet but quality control parameters on liquid diet were generally lower compared with the insects that were maintained on whole mango fruit and carrot diet supplemented with mango powder.
- Feeding adult *C. cosyra* with the standard enzymatic yeast hydrolysate diet resulted in significantly higher egg production compared with adult flies fed on the local Kenyan yeast.

1.6. *Ceratitis fasciventris*, *C. rosa* and *C. anonae*

- Several oviposition devices were tested for egg collection in *C. fasciventris* and the plastic bottle was found to be the most effective.
- Both sugarcane bagasse and carrot based diet was effective in larval rearing of *C. fasciventris*.
- Demographic parameters on *C. fasciventris* on carrot based diet were documented.
- *Ceratitis fasciventris* was able to successfully develop on the liquid diet although quality control parameters were generally lower compared with carrot-based diet.
- Adult feeding on enzymatic yeast hydrolysate produced more eggs and had higher egg hatch than those fed on local yeast.
- Fruit juices of *Monodora grandidieri* elicit oviposition simplifying egg collection process of *C. rosa*.
- *Ceratitis rosa* readily accepted larval diets based on carrot, wheat and the standard liquid diet for larval development.
- In *C. anonae*, fruit juices of several host plants of the insect failed to elicit oviposition in the insect. This species continues to be maintained on whole mango fruit at the *icipe* rearing facility.

1.7. *Dacus ciliatus*

- The CRP provided the ground to develop the first elements for the artificial rearing of the fly
- Several larval diets were tested and a semi-liquid larval diet selected for future enhancements
- A prototype for an egging adult cage was developed and tested
- Egging systems, which include egg-laying stimulants, were tested and adopted
- Conventional adult diets for other fruit flies were modify and adapted to *D. ciliatus*
- Phagostimulation in adult flies was investigated and strong phagostimulants detected

2. Species in which Key Mass Rearing, Quality, and Handling Bottlenecks Need to be Overcome

2.1. *Anastrepha serpentina*

- An oviposition device to collect 0.5 million eggs/cage per day was developed along with double oviposition panel adult cages holding 80,000 flies.
- The larval diet used for mass rearing of *A. ludens* was tested and gave good results. Optimal larval density (greatest larval weight) was found to be 3 larvae/gram.
- Naked pupation was found to reduce mortality caused by larval aggregation.
- Demographic, rearing and quality parameters were determined in order to assess mass-production potential.
- Potentially, *A.serpentina* production could reach 50 million a week within 2 generations.

2.2. *Anastrepha striata*

- A dome oviposition device was developed in order to collect 30 eggs/female/day.
- A larval diet was developed, showing that a density of 2 larvae/gram of diet the use of a starter larval consisting of corn, rice, potato starch, torula yeast and sugar gave best results. *A. striata* was found to be sensitive to nipagin.
- Naked pupation was used to avoid mortality caused by larval aggregation.
- Demographic, rearing, and quality parameters were determined in order to define mass-production potential.
- For first time a colony under 100% artificial conditions was established (without need of host fruit for oviposition) however development of mass rearing for this species requires substantial improvement of oviposition devices.

2.3. *Anastrepha fraterculus*

Argentina

- Larval diet has been improved to rear *A. fraterculus* in Tucumán. The new formula improved egg-pupae recover by using sponge cloth as an alternative to agar, double amount of sugar and pH 4.0. It is yet necessary to determine water content and to evaluate the adequate amounts of ascorbic acid in complementation with citric acid that rendered in better performance. Result should be confirmed at a higher scale. Sugar intake was stopped when protein intake reached its “optimal” value. It seems necessary to deliver protein in conjunction with phagostimulants (sugar). A second step will be to evaluate the diet at a larger scale and determine the feasibility to reuse the sponge cloth to reduce costs.
- Experiments of survival and dispersal indicated that adult diets composed of high proportion of protein are associated to reduced resistance to starvation conditions both in laboratory and field cages. The ability to survive in the field also is affected negatively by high proportion of protein in the diet. The dispersal patterns are not modified by adult diet, but survival of flies fed on sugar only seems to be increased respect to diets with proteins. The study suggests that it is important to evaluate the optimal proportion of sugar:protein in adult diet to get the best compromise between survival and optimal sexual development.
- Comparison of the phenotype for multiple traits between wild flies and the laboratory strain of Estacion Experimental Obispo Colombres, Tucuman, indicated a significant morphometric differentiation accompanying adaptation to lab conditions. The consequences of such effect should be analysed in terms of efficiency of released flies in SIT based programs.

Brazil

- A colony of *A. fraterculus* and a colony of *A. zenildae* were established at the lab of EMBRAPA, Agroindustria Tropical. Mating compatibility between these species was analysed with populations from Ceara, Rio Grande do Norte and Sao Paulo.
- A colony of *A. fraterculus* was established in the Radioentomology Laboratory of University of Sao Paulo. The species was identified as *A. sp1 aff. fraterculus* according to Dr. Selivon description.
- The colony has been stabilized and nowadays it has reached the 29th generation. The original protein source, imported yeast hydrolysed, was substituted by a much cheaper local yeast extract with excellent results.
- The rearing of the parasitoid *Diachasmimorpha longicaudata* on *A. fraterculus* larvae has been developed.

2.4. *Bactrocera oleae*

- Artificial rearing was improved throughout the CRP
- The antibiotics were removed from the adult diet without detrimental effects
- Probiotic adult diets based on associated bacteria of olive fly were developed
- A successful hybrid strain ("Argov") for SIT pilot applications in Israel was developed
- A solid starter-liquid finisher (without cellulose) larval diet was tested and found promising
- Evidence for horizontal transference of endosymbionts was detected in the "Argov" strain
- The CRP demonstrated that it is essential for successful and easier colonization to use field collected adults instead of larvae derived from field collected infested fruit
- The CRP demonstrated that the composition of the associated bacterial community is determined by the type of host variety and maturation stage, geographic source of host and artificial diet
- The CRP provided the basis to enhance our knowledge on the symbiosis between the olive fly and *Candidatus* Erwinia dacicola
- The CRP served as a springboard for Israel's pilot SIT, which is currently being implemented

2.5. *Bactrocera zonata*

Pakistan

- The larval diet of laboratory reared *B. zonata* was improved by the use of Torula yeast in place of protein hydrolysate, brewers' yeast or Nu-Lure® based on quality parameters such as % pupal recovery, % adult eclosion, % fliers and % egg hatch.

Mauritius

- Oviposition stimulants - Results showed that water was equally effective as an oviposition stimulant as papaya, mango, peach and guava juices
- Egg production: 90% of eggs were produced within the first three weeks post adult eclosion and averaged at 52 eggs per female over this time.
- Larval diets: A new diet based on sugar cane bagasse and omitting coarse and ground maize was found to improve or maintain all tested quality parameters over at least 4 generations.
- Liquid larval diets: Results over two generations show high potential for the USDA liquid diet but needs to be tested on a mass-rearing scale.

3. Species with Ongoing SIT where Improved Mass Rearing and Process Control is Needed

3.1. *Anastrepha ludens*

- From a mass rearing perspective a liquid diet was tested with good results but with yields inferior to solid diets in Metapa. Several sources of cheap protein for adult food were tested. However ICN was kept as the best component of adult diet.

- Substantial evidence was gathered in the course of the CRP demonstrating that doses of 8 Krads applied to sterilize 100% of males are excessive and affect male mating performance, greater sterility induction and mating performance was achieved by males irradiated at low doses causing 100% female sterility. Doses between 5-6 Krads can be safely applied to maximize male mating performance and sterility induction in the field.
- The *A.ludens* strain was found to be partially isolated from wild populations, a scheme of strain refreshment through hybridization of wild males and lab females restored male mating competitiveness in the F1 generation.
- Male performance of hybrid males remains to be tested for more than five generations.
- Experiments of survival and dispersal indicated that adult diets composed of high proportion of protein are associated to reduced resistance to starvation conditions both in laboratory and field cages. The ability to survive in the field also is affected negatively by high proportion of protein in the diet. The dispersal patterns are not modified by adult diet, but survival of flies fed on sugar only seems to be increased respect to diets with proteins. The study suggests that it is important to evaluate the optimal proportion of sugar:protein in adult diet to get the best compromise between survival and optimal sexual development.

3.2. *Anastrepha obliqua*

- A Modified liquid diet for *A.obliqua* did not improve yield of the standard solid diet used in Metapa.
- Adult density in colony cages and larval density trials revealed that a reduction from 100,000 to 60,000 adults per cage and a reduction from 6 to 3 larvae/g of diet resulted in increased egg to pupae conversion and an increase in pupal weight. These parameters were adopted in mass rearing in Metapa where weekly production is 50 million.
- As for *A. ludens* greater sterility induction and increased male mating performance was achieved by males irradiated at low doses than by males irradiated at the standard 8 Krad dose. A dose of 4 Krad causing 100% female sterility and 99% male sterility can be safely applied to *A. obliqua* for release in areas of pest prevalence.
- A colony was established in Brazil and has now been held under artificial conditions for 12 generations.
- Experiments of survival and dispersal indicated that adult diets composed of high proportion of protein are associated to reduced resistance to starvation conditions both in laboratory and field cages. The ability to survive in the field also is affected negatively by high proportion of protein in the diet. The dispersal patterns are not modified by adult diet, but survival of flies fed on sugar only seems to be increased respect to diets with proteins. The study suggests that it is important to evaluate the optimal proportion of sugar:protein in adult diet to get the best compromise between survival and optimal sexual development.

3.3. *Bactrocera cucurbitae*

Sri Lanka

- Pupation medium: Sand was better than sawdust or soil and cheaper than the standard vermiculite.
- Adult diet: A local protein bait was found to be promising as an alternative to the more costly standard protein. Needs more testing to confirm.

Bangladesh

- Development of larval and adult diets: Quality analysis of the flies reared on diets showed that a new semi-solid diet (replacing rice bran with mill feed) was optimal over the others tested and is now being used routinely in Bangladesh. An adult diet of casein: yeast extract: sugar (1:1:2) appeared most efficient over those tested.
- Sterility dose determination: A dose of 50Gy is required to sterile males when pupae are irradiated at 120h post-pupation.

- Mating competitiveness of the irradiated male flies: Irradiated males (50Gy) were as competitive in mating as those of wild, non-irradiated males.
- Ratios of irradiated males over non-irradiated males to suppress the population in lab and field cages: A ratio of irradiated males to non-irradiated males of 9:1 reduced populations in laboratory and field cage trials by about 85%.

3.4. *Bactrocera dorsalis*

- Liquid larval diet ingredients such as wheat germ oil, brewer's yeast, streptomycin, citric acid, were evaluated and proved to be able to be replaced with cheaper, more easily available alternatives such as corn oil, torula yeast, tetracycline, and HCL. Mass rearing using liquid diet needs to be encouraged and implemented

3.5. *Bactrocera philippinensis*

- New adult screen cages using perforated egging tubes have been designed for *B. philippinensis*.
- The replacement of sweet potato with sugarcane bagasse, rice straw and powdered corn as an alternative bulking agent in the standard artificial diet show positive results in large scale rearing.
- Rearing protocols and product quality control procedures in egg incubation, sieving of pupae and pupal holding procedures were established.
- The USDA larval liquid diet was successfully tested for *B. philippinensis* in terms of pupal weight, % emergence, % fliers, sex ratio, female fecundity and fertility. Based on the criteria for screening and development of the liquid diet, the insect performed most satisfactorily when corn oil was added to the liquid medium.

3.6. *Bactrocera tryoni*

- A finding that the addition of common enterobacteria to *B. tryoni* larval diets did not increase the fecundity of newly domesticated females.
- A model accounting for the main features of the loss of genetic variability during the domestication of *B. tryoni*.
- A recommendation that mass rearing lines be regularly re-derived from the hybridization of 4 or more inbred lines.

(b) Others:

- **Collaboration between laboratories**

- Collaboration between the Insect Biotechnology Division of Bangladesh Atomic Energy Commission and the USDA-ARS, Hawaii, with the Entomology division of Bangladesh Agricultural Research Institute and with a private entrepreneur Safe Agriculture Bangladesh Limited
- The USDA-ARS, Hawaii collaborated with the following institutions, namely Ministry of Agro Industry, Food Production & Security, Mauritius, the Philippine Nuclear Research Institute, Philippines, ICIPE, Nairobi, Kenya, Thailand, China, South Africa, Spain, Guatemala, Slovakia, and Argentina, for the development of the liquid larval diet.
- In Mexico various Scientific Discussion Groups were set up to develop and plan studies and to discuss methods and results:
  - El Colegio de la Frontera Sur, Programa Moscafrut and Universidad Autónoma de Chiapas (Centro de BioCiencias): Development of an artificial larval diet for *Anastrepha striata* and improvement of artificial rearing methods for *Anastrepha serpentina.*
  - Programa Moscafrut and US Pacific Basin USDA-ARS: Development of a liquid larval diet for *Anastrepha* species.

- Instituto de Ecología A.C., Programa Moscafrut, and Universidad Veracruzana: Evaluation of reduced irradiation doses on male mating performance and sterility induction of *A. ludens* and *A. obliqua*.
- ECOSUR, Mexico and the Laboratorio Genética de Poblaciones Aplicada Argentina: Survival and dispersal of *A. ludens* and *A. obliqua*
- Estación Experimental Agroindustrial Obispo Colombres (Tucumán, Argentina), Dept. of Entomology, Institute of Plant Protection, ARO, The Volcani Center (Beit-Dagan, Israel), U. S. Pacific Basin Agricultural Research Center, USDA-ARS (Hawaii, USA), and Department of Agriculture, Crop Production and Rural Environment, University of Thessaly (Greece): Artificial rearing of *A. fraterculus*
- Centre for Nuclear Energy in Agriculture, USP, Piracicaba, (SP, Brazil), Biosciences Institute, USP, São Paulo, (SP, Brazil), Embrapa Semi-Arid, Petrolina, (PE, Brazil), and Estación Experimental Agroindustrial Obispo Colombres (Tucumán, Argentina): Development of artificial rearing for *A. fraterculus.*
- Laboratorio Genética de Poblaciones Aplicada, Argentina, Laboratorio de Genética de Insectos de Importancia Económica, Argentina, and Estación Experimental Agroindustrial Obispo Colombres, Argentina: Morphometric trait differentiation between wild and a mass reared populations of *A. fraterculus* and survival and dispersal of *A. fraterculus*.

- **Capacity building**

- The CRP contributed to the formation of human resources and establishment of research groups in each of the 17 countries originally included.
- Capacity for fruit fly rearing in the Insect Biotechnology Division Bangladesh has increased in terms of space, maintaining temperature and humidity control system. But more space will be required for near future test field release programme of SIT
- A new building was constructed for the mass rearing of both *B. zonata* and *B. cucurbitae* in Mauritius.
- The mass-rearing facility in Stellenbosch, South Africa was extended to include liquid larval diet rearing.
- An Olive fly SIT pilot trial in Israel was initiated within this CRP which served as a support and source of experts.

- **Fellowships, PhDs, study tours, etc**

- Two fellows from Seychelles visited the Mauritius laboratory in 2009 to study the rearing of *B. zonata* and *B. cucurbitae.*
- Officers from Mauritius visited the fruit fly rearing laboratories in Hawaii, Philippines and Thailand.
- Two fellows from Mauritius, three fellows from Florence, Italy, and one fellow from Biotechnical Institute of Norman Boulaug Institute were trained in USDA-ARS-Honolulu in fruit fly nutrition in 2008.
- One PhD and one MSc student are associated with the fruit fly SIT research programme on *B. cucurbitae* in Bangladesh.
- MS students from Universities in Pakistan visited the laboratory to learn rearing techniques of fruit flies.
- A workshop was organised on biocontrol and mass rearing of fruit flies besides other pests in Pakistan.
- A fellowship from the Secretaría de Relaciones Exteriores (SRE) of México allowed a scientific visit of Maria Eugenia Utgés from the Lab. Genética de Poblaciones Aplicada (GPA, EGE, FCEyN, UBA, Buenos Aires, Argentina) to the Laboratorio Ecología de Poblaciones de Moscas de la Fruta (ECOSUR, Tapachula, Chiapas, México).

- The CRP contributed to the formation of several fellows and students, among which Paula Gómez Cendra obtained her PhD, and Andrea Oviedo and Thiago de Araújo Mastrangelo their MSc degrees.
- Abraham Barreda-Landa and Laura Paulin obtained their BSc and Licenciatura (respectively) on experiments in this CRP.

▪ **International attention on fruit fly species other than *Ceratitis capitata***

- Sotero S. Resilva from the Philippines conducted mission assignment to study pupation process and recorded eye colour changes of pupae at different holding temperature such as *Bactrocera zonata* and *B. cucurbitae* in Mauritius, *Anastrepha ludens*, *A. obliqua*, and *A. serpentina* in Mexico, *B. doralis*, *B. invadens*, *B. cucurbitae* (GSS), *B. oleae*, *B. tryoni* and *A. fraterculus* in Seibersdorf, Austria.

▪ **Technology transfer**

- Fruit fly liquid larval diet technology was evaluated by participants from 36 countries for at least 24 species. There were at least 11 countries was successfully demonstrated and used in their countries. These include Bangladesh (*B. Cucurbitae* and *B. dorsalis*), Philippines (*B. philippinensis*), Mauritius (*B. zonata* and *B. cucurbitae*), China (*B. dorsalis*), Thailand (*B. correcta*), Kenya (*B. invandens*, C. rosa), Argentina (*Anastrepha fraterculus*).
- Fruit fly liquid larval diet technology was also transferred to medfly mass rearing facilities worldwide including CDFA Medfly mass rearing facility in Waimanalo, Hawaii; Stellenbosch Medfly rearing facility in Stellenbosch, South Africa, El Pino Medfly fly rearing facility in Guatemala, and Caudete Medfly rearing facility in Valencia, Spain.
- Transfer male annihilation technology to the growers for the management of fruit flies, *B. zonata* in Pakistan

Effectiveness of CRP:

1. (a) In reaching Specific Objective:

1. To improve/develop mass-rearing diets used in rearing of selected *Anastrepha and Bactrocera* fruit fly pests.

Artificial larval and adult diets were formulated and developed, modified and tested on 20 species of pest fruit flies and evaluations and recommendations made. Advances have been achieved on the development of the liquid diet to suit locally available ingredients and materials. Advantages and/or limitations of the addition of protein to adult diets were evaluated in terms of development, survival, competitiveness and dispersal.

2. To assess the impact of biotic and abiotic factors on production and quality of mass-reared fruit flies.

Probiotic bacterial formulations were evaluated on some pest fruit fly. Progress was made in identifying symbionts and associated bacterial communities and the environmental factors affecting the composition of the communities and their importance in rearing and adult release. Recommendations to assess using wild adults and a combination of hybridization between lines to initiate and maintain lab colonies were made. Strategies to avoid or minimize the use of antibiotics and preservatives were assessed and recommendations made.

Suitable temperature, relative humidity and other environmental physical conditions were determined for the establishment of new laboratory cultures of pest fruit flies.

Physical and chemical systems to stimulate egg laying were identified and assessed. Recommendations were made to improve egging devices, cage design and pupal substrates.

3. To increase the efficiency and cost effectiveness of mass-rearing fruit flies for SIT.

Strategies to avoid inbreeding and its effects on male mating performance were identified. Optimization and simplification of diet ingredients using cheaper, locally available ingredients have been achieved and implemented for different pest fruit fly species.

4. To develop improved handling, process control and automation of mass-reared fruit flies.

Sterilization doses for several species were revised and new recommendation to lower irradiation doses were issued. Reduction of space and labor requirements resulted from the adoption of simplified rearing systems. Increase biosecurity resulted from new rearing methodologies. The CRP discussed strategies to develop automation in the future.

(b) In contributing towards Overall (i.e. Agency Project) Objective:

To develop and improve rearing technology for selected *Anastrepha and Bactrocera* fruit flies of economic importance for SIT application.

The CRP successfully reached the following stages of development towards SIT for the following fruit fly categories (in terms of starting point):

(1) Regarding basic biology and colonization parameters the group produced information for 7 poorly known emerging species of pestiferous fruit flies

(2) In terms of achieving artificial breeding, the CRP successfully managed to develop artificial egg-laying systems for 8 emerging pest species of fruit flies

(3) The CRP was able to adapt 8 species of emerging pestiferous fruit flies into artificial larval and adult development diets for mass-rearing and SIT, and mass rearing of two parasitoids for biological control purposes

(4) Production parameters for 4 species of pestiferous fruit flies were brought to the level suitable for mass production during the CRP

(5) The CRP succeeded on improving the mass-rearing technologies for at least 5 of pest fruit flies

(c) Factors, if any, which adversely affected the effectiveness of the CRP:

i. *Appropriateness of the CRP*: The CRP and all topics addressed during the CRP were appropriate, and there was good communication and interaction among participants. No adverse factors affecting the appropriateness of the CRP were encountered.

ii. *Formulation of the CRP:* The CRP was properly formulated following the recommendations of a consultants meeting that used specific criteria to select the best set of Tephritidae pest as a candidate for the further development of the SIT as a control tactic.

iii. *Management problems during the implementation of the CRP:* No significant management problems were encountered.

iv. *Intellectual, financial, and other inputs from participants:* Considerable inputs, both intellectual and in-kind, were provided by the participants of the CRP and the institutions

that hosted the RCMs.

Impact of the CRP:

1. Demonstrated potential of rearing and mass producing newly emerging pestiferous fruit flies.
2. Established the basis for improving mass rearing of pest fruit flies having basic unsolved problems and upgraded the knowledge and methodologies for ongoing mass-reared fruit flies
3. Gained relevant knowledge and know-how on behaviour and ecology of poorly known pestiferous fruit flies
4. Initiated transfer of technology to the private sector for mass production
5. Enhanced worldwide networking and collaboration among and between researchers in mass rearing.
6. Increased awareness of the growers and policy makers of the benefits of the SIT.

Relevance of the CRP:

1. The CRP contributed to the global expansion of SIT as an environmentally-friendly, area-wide IPM system for pestiferous fruit flies.
2. Quality control parameters, including dispersal and survival of fruit flies, have been established as important aspects for optimization of mass-rearing fruit flies for SIT.
3. The number and frequency of biological invasions by emerging pest species such as *Bactrocera zonata* in Africa renders the development of mass-rearing and ST-related technology for these species a highly relevant issue.
4. The CRP contributed to more efficient laboratory establishment and mass-production of pest fruit flies suitable for SIT programmes in area-wide IPM programmes. Further, it provided fundamental knowledge for future development in the mass-rearing of new pest fruit fly species and optimized the cost effective use of SIT for ongoing programs.

Recommended future action by Agency:

(1) Continued support for research and development on potentially invasive and expanding pest fruit fly species.
(2) Expand the capabilities of regions to develop mass-rearing and SIT programmes for invading and expanding fruit fly species such as *Bactrocera invadens*.
(3) In order to develop more efficient larval rearing media for problematic species in terms of SIT development support of research on the role of symbionts and their management in diets should be provided.
(4) Support should be provided to further understanding of factors causing periodic colony crashes in species such as *Anastrepha fraterculus, A. striata, Bactrocera oleae, B. pyrifoliae*.
(5) Consideration of colonising diapausing pest fruit fly species in support of SIT, for example, *Rhagoletis spp* and *Bactrocera minax*.
(6) More emphasis should be given to the incorporation of genetic exploration and molecular biology as tools to better understand and develop effective colonisation and colony maintenance. A better understanding of the effects of colonisation and mass-rearing on the genetics and consequent effects of SIT efficiency and efficacy should be encouraged.
(7) Stimulate contact between experienced and new breeders of specific fruit fly species in order to avoid duplication of efforts.

(8) To incorporate nutrigenomics and molecular biology into studies to understand and improve diets and dietary intake of mass-reared fruit flies.

Resulting Publications:

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Chang, C. L. 2009. Fruit fly liquid larval diet technology transfer and update. J. Appl. Entomol., 133: 164-173.

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