Title of the Co-ordinated Research Project:

Improving Sterile Male Performance in Fruit Fly Sterile Insect Technique (SIT) Programmes

Section/Division: IPC/NAFA-NAAL

Project Officer: HENDRICHs Jorge

Period Covered: 2004-07-01 through 2009-12-31

Objectives of CRP:

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

The overall objective of this CRP was to ultimately reduce the cost and increase the effectiveness of SIT programmes by improving the performance of mass produced sterile males in operational fruit fly SIT programmes, specifically through manipulations implemented at the emergence and release facilities. The ultimate goal is to assist Member States in achieving sustainable fruit and vegetable production and in facilitating trade by applying area-wide SIT suppression/eradication programmes against fruit flies of economic importance.

(b) Specific (CRP):

1. For selected Anastrepha, Bactrocera, Ceratitis spp. assess the effects of hormone/analogue supplements, and develop hormone supplements procedures for incorporation into pre-release protocols for ongoing Anastrepha spp. SIT programmes. In particular:

1.1. Determine under standard conditions, if unknown, the age at which wild and sterile males become sexually mature.
1.2. Assess if juvenile hormone (JH) analogue treatment (or other conditions that stimulate JH production) significantly improves reproductive maturation, sexual performance and physiological parameters.
1.3. Determine the optimal dose required.
1.4. Assess the interaction of irradiation, nutritional and other hormone supplements on sexual maturity in the laboratory.
1.5. Compare mating success of hormone and protein-fed and protein-deprived sterile males competing with wild males for wild females using standard field cage protocols.
1.6. Develop delivery systems other than topical application and assessment of efficacy under laboratory conditions.
1.7. Develop efficient large-scale methods for incorporation of JH analogues.
1.8. Conduct of pilot scale tests that allow assessment of the method.
2. For Anastrepha spp., Bactrocera spp. and Ceratitis spp.: Conduct basic research on adult diets and their effect on sexual performance of wild and sterile males. In particular:

2.1 Assess natural foods
2.2 Characterize the diet that optimizes male sexual performance
2.3 Assess the interaction between diet sexual performance, dispersal and survival
2.4 Identify the optimal formulation/delivery system and presentation in which diet is made available to sterile males, including the evaluation of inexpensive, locally available sources of nutrients.
2.5 Study the effects of mass rearing conditions and irradiation on gut physical and biochemical integrity.
2.6 Assess the contribution of microorganisms to fly health and sexual performance, and investigate effects of inoculating sterile males with microorganisms on survival and copulatory success.

3. For selected Anastrepha, Bactrocera; Ceratitis spp. assess the effects of semiochemical supplements, including natural sources, and develop delivery systems for exposure of methyl-eugenol (ME), and essential oils and other natural products to sterile males. In particular:

3.1. Search for semiochemical compounds that affect *Anastrepha* spp. male sexual performance.
3.2. Assess various semiochemicals in terms of enhancing *Bactrocera* spp. sterile male performance.
3.3. Determine the optimal dose of ME/CUE that does not kill *Bactrocera* spp. male flies and increases their sexual performance.
3.4. Determine the optimal feeding age, and time and duration of exposure, as well as the interactions between feeding on ME/CUE, hormone treatment and/or nutrition in terms of *Bactrocera* spp. male competitiveness.
3.5. Study the behavioural, physiological and ecological effects of *Ceratitis* spp. exposure to semiochemical supplements.
3.6. Identify the chemical composition, including active compounds involved in *Ceratitis* spp., their modes of action, and comparison of their effects.
3.7. Compare different semiochemical sources (both natural and commercial) in terms of their effect on *Ceratitis* spp. mating performance.
3.8. Identify an optimal delivery system and protocols to make these substances available in *Ceratitis* spp. SIT programmes.
3.9. Assess the cost-benefits of incorporating these supplements into *Ceratitis* spp. SIT operations.

4. For selected *Anastrepha*, *Bactrocera*; *Ceratitis* spp. manipulate fly holding conditions at the fly emergence, holding and release centres to significantly improve male performance in the field, and assess fly release conditions (e.g. holding temperature, fly immobilization, fly density, fly handling and release system) for effects on male performance. In particular:

4.1. Compile all current procedures to ship, emerge, feed, hold, knockdown and release sterile flies.
4.2. Assess the effect of varying relevant environmental parameters, such as temperature, relative humidity, and illumination on sterile male performance.
4.3. Compare the effects of different emergence systems, including varying the size (volume) of the containers and resulting fly density, on sterile male performance in the field.
4.4. Assess the interaction of different holding conditions and hormonal, nutritional and/or semiochemical supplements, on sterile fly performance.
4.5. Compare males resulting from standard conditions with males preconditioned to specific field conditions, such as high or low temperature, light cycle, light intensity, or elevation.
4.6. Design of fly holding systems to optimize the engineering and automation to improve SIT efficiency through reduction of construction and operational costs in order to improve fly quality at release.
4.7. Assess the effects of cold knockdown for immobilizing the flies on their performance, particularly for cold-sensitive species, and investigating alternatives if required.
4.8. Assess the effects on fly performance of different sterile fly release systems, as well as the interaction with hormonal, nutritional and/or semiochemical supplements.

OUTPUTS:

(a) Research:

1. HORMONAL SUPPLEMENTS

1.1. Determination under standard conditions, if unknown, of the age at which wild and sterile males become sexually mature.

Studies have determined the age at which laboratory reared and wild males of *Anastrepha fraterculus*, *A. ludens*, *A. obliqua*, *A. suspensa*, *A. serpentina*, *A. striata*, *Bactrocera correcta*, *B. dorsalis*, *B. philippinensis*, *B. tryoni*, *B. cucurbitae*, *Ceratitis capitata*, and *C. rosa* become sexually mature. This information has been used as the basis on which efficacy of hormone treatments in accelerating reproductive development.

1.2. Assessment if juvenile hormone (JH) analogue treatment (or other conditions that stimulate JH production) significantly improves reproductive maturation, sexual performance and physiological parameters.

Research has documented that hormone therapy alone has a positive effect on accelerating reproductive development in *Anastrepha fraterculus*, *A. ludens*, *A. obliqua*, *A. suspensa*, *A. serpentina*, *Bactrocera cucurbitae* and *B. tryoni*. However, combination of hormone therapy with an adult diet containing also protein is at least twice as effective as hormone therapy alone for these species. Research also documented that hormone therapy had no effect on either *Ceratitis capitata* or *Bactrocera dorsalis*. The reasons for this appear to be that laboratory strains of *C. capitata* become sexually mature much faster than other species for which hormone therapy has an effect. For *B. dorsalis* pheromone biosynthesis and reproductive maturity requires sequestration of ME from plant sources so hormone therapy alone does not impact reproductive development.

1.3. Determination of optimal dose required to accelerate maturation.

The optimal dose of hormone has been determined for *A. suspensa*, *A. ludens*, *A. fraterculus*, *A. obliqua* and *B. cucurbitae*. Topical applications of 2.5 or 5ug of methoprene are effective for all species. Additional research has demonstrated that large scale dipping of pupae in acetone baths containing 0.05% methoprene does not impact emergence or survival of adult flies and accelerates reproductive development as well as or better than topical application of methoprene to individual flies. Blocks containing agar, 5-10% protein and 0.05% methoprene
effectively accelerated development of male *A. suspensa*. A diet containing sugar, protein and 0.0015% methoprene, developed as a pre-release diet for operational SIT programmes for *A. ludens* and *A. obliqua*, has been shown to effectively accelerate development for these species.

1.4. Assessment of interaction of irradiation, nutritional and other hormone supplements on sexual maturity in the laboratory.

Assessments of interaction of irradiation, nutritional and other hormone supplements on sexual maturity in the laboratory have demonstrated that the supplements have no negative effects on males of *A. fraterculus, A. ludens, A. obliqua, A. suspensa,* and *B. tryoni*. Indeed, for all of these species application of methoprene resulted in acceleration of reproductive maturity in the same way that it does in non-irradiated males.

1.5. Comparison of mating success of hormone and protein-fed and protein-deprived sterile males competing with wild males for wild females using standard field cage protocols.

Studies comparing mating success of hormone and protein treated and untreated sterile males competing with wild males for wild females using standard field cage protocols have been performed using *A. fraterculus A. ludens* and *A. suspensa*. In all cases irradiated males treated with hormone and fed sugar plus protein effectively competed with wild males but at significantly earlier ages.

1.6. Development of delivery systems other than topical application and assessment of efficacy under laboratory conditions.

Several systems for delivery of methoprene have been developed and assessed. These include: an agar based diet containing 5 - 10% protein along with 0.05% methoprene tested with *A. suspensa*; a technique in which pupae are dipped in an acetone bath containing 0.05% methoprene tested with *A. fraterculus, A. ludens* and *A. suspensa*; and a sugar protein paste made by mixing 1 kg of dry diet with 1 litre of water containing 0.0015% methoprene and then painting this onto paper which is added to pre-release holding cages and tested in operational SIT programmes for *A. ludens* and *A. obliqua*. Although all of the diets effectively accelerated reproductive development, the agar based diet resulted in large amounts of waste and was not cost effective. The acetone dipping method was actually more effective than topical application of methoprene to individual flies, but it is impractical because of danger, safety and disposal issues associated with the use of acetone. Thus, the most effective delivery system is sugar/protein/methoprene paste, which is now being used in operational programmes.

1.7. Development of efficient large-scale methods for incorporation of JH analogues.

Results from development of formulations of hormone and protein delivery systems have been accepted by an action programme, Moscafrut, and are now incorporated into protocols for delivery/release of two species, *A. ludens* and *A. obliqua* in Mexico.

1.8. Conduct of pilot scale tests that allow assessment of the method.

Hormone treated and untreated *A. ludens* were released in two 3,000 ha areas in the north of Mexico in a pilot test. The dispersal and recapture of treated and untreated flies was similar. In addition, in the area with treated flies, neither trapping showed wild fly capture nor did fruit sampling indicate larval present in fruit at the end of the large field test. Based on these
results the Mexican Fruit Fly campaign now routinely employs hormone therapy and protein in pre-release holding protocols for SIT programmes involving *A. ludens* and *A. obliqua*.

2. **NUTRITIONAL SUPPLEMENTS**

2.1. *Assess natural food and their effect on sexual performance of fruit fly males.*

The importance of a fruit diet for copulatory success was established for *A. ludens* and *A. obliqua*. Males fed on orange or mango exhibited improved sexual performance. In both species pheromone production is quantitatively affected by diet.

2.2. *Characterize the diet that optimizes male sexual performance.*

In species representing all three genera the diet that optimizes male sexual performance has been characterized. This includes *C. capitata*, *A. suspensa*, *A. ludens*, *A. obliqua*, *A. fraterculus*, *B. tryoni*, *B. zonata*, *B. correcta*, *B. cucurbitae* and *B. philippinensis*. In all these species, addition of protein to the diet significantly improved sexual performance.

In several species, studies indicate that the quality and amount of the nitrogenous diet and the age of ingestion by sterile males may significantly affect SIT outcomes. In *A. ludens*, *A. obliqua* and *A. fraterculus*, and *B. tryoni*, the ratio of protein to carbohydrate in dry diets provisioned early in adult life contributed to male performance and survival in a dose dependent manner. In *B. cucurbitae* and *C. rosa* addition of protein into the diet significantly enhanced male sexual performance. Observations on *B. philippinensis*, *B. correcta* and *B. dorsalis* showed that addition of protein to the diet improves male performance.

2.3. *Assess the interaction between diet sexual performance, dispersal and survival.*

Progress was made in understanding the interactions between diet, sexual performance, survival and dispersal. In general, protein enriched diets improved sexual performance. Furthermore, correlates between diet and sexual performance were shown in *C. capitata*, *A. suspensa*, *A. ludens*, *B. dorsalis*, *B. correcta*, *B. cucurbitae*, *B. tryoni*, and *B. philippinensis*. Links between diet and survival were demonstrated in *B. tryoni*, *A. fraterculus*, *A. suspensa*, *A. ludens* *A. obliqua* and *C. capitata*.

Sterile male *A. ludens*, *A. fraterculus* and *A. obliqua*, fed on protein rich diets, had a lower resistance to starvation, were less likely to be recaptured in protein baited traps or to respond to bait applications, and may have moved less and died faster than only sugar-fed counterparts. Several studies on *C. capitata* and *B. tryoni* suggest that protein rich post-teneral diets do not adversely affect sterile male survival and movement in the field. In laboratory studies, *B. cucurbitae* and *B. tryoni* males fed also protein lived significantly longer than only sugar-fed males. Reducing the amount of protein in the pre-release diet showed a favourable effect on survival and dispersal of *A. ludens*, *A. obliqua* and *A. fraterculus*.

Another series of studies demonstrated that the ability of sterile males to inhibit female remating was significantly improved by feeding on a nitrogen rich diet (*A. fraterculus*, *B. tryoni*, and *C. capitata*).

2.4. *Identify the optimal formulation/delivery system and presentation in which diet is made available to sterile males, including the evaluation of inexpensive, locally available sources of nutrients.*
A formulation based on soy whey protein and incorporating methoprene has been developed and tested for *A. suspensa*. Concurrently, a commercial product ("Mubarqui") has been introduced into release programmes for *A. ludens* and *A. obliqua*.

Overall, local sources of protein hydrolysates were not found to be better than industrial brands commonly used. However, the beneficial effect of protein may be achieved with a relatively small amount, provided to males early in adult life. For example, in *B. tryoni*, *A. fraterculus*, *A. ludens* and *A. obliqua*, as little as 4-9% protein in the diet sufficed to significantly enhance male sexual performance.

2.5. Study the effects of mass rearing conditions and irradiation on gut physical and biochemical integrity.

Although irradiation is known to be detrimental to the fly gut wall, irradiation did not affect the activity of proteases and peptidases in the gut of male *C. capitata*. In *B. tryoni*, irradiation significantly diminishes the ability of the flies to tolerate protein deprivation.

2.6. Assess the contribution of microorganisms to fly health and sexual performance, and investigate effects of inoculating sterile males with microorganisms on survival and copulatory success.

Composition and function of the microflora of the adult gut has been described in *C. capitata*. In addition, it was found that the diversity of the microflora of laboratory reared and irradiated males are low compared to wild males. Concurrently, progress has been made in understanding the microflora of *B. oleae*. *Candidatus Erwinia dacicola* appears to be the main symbiont, inhabiting the oesophageal valve and anal diverticula of flies. Other bacteria inhabit the gut.

The contribution of microorganisms to fitness of non-sterile *C. capitata* was established. Studies on the effects of manipulating the microflora of sterile males on male performance suggest that probiotic supplements could improve male sexual performance (*B. oleae* and *C. capitata*).

3. SEMIOCHEMICAL SUPPLEMENTS

3.1. Search for semiochemical compounds that affect Anastrepha spp. male sexual performance.

An effort to determine the effect of orange and mango fruit on male mating competitiveness of *A. ludens* in field cages was conducted. In addition, gas-chromatography/ mass spectrometry (GC-MS) analyses of pheromone compounds have been carried out from males exposed to these different sources. Screening several natural compounds has resulted in finding an effective attractant for *A. obliqua*. Studies on *A. fraterculus* have been conducted to evaluate the effect of exposure of males to different fruit including guava, lemon, grapefruit, sweet orange, mango, and papayas on male mating performance. Exposure to guava volatiles resulted in a positive effect on male mating performance. Experiments have been conducted to determine the best exposure times (1, 5, 10 and 15 days) to guava fruits without determining an optimal time. The impact of exposure of *A. fraterculus* to ginger root oil (GRO) was evaluated and it was found to enhance male mating performance; however, some of the tests resulted in detrimental effect. Similar results were obtained for exposure to lemon fruit volatiles.
3.2. Assessment of various semiochemicals in terms of enhancing Bactrocera spp. sterile male performance.

Holy and sweet basil were found to be natural sources of ME. In *B. philippinensis* exposure to basil oil had a positive effect on male mating performance. However, for *B. dorsalis* and *B. correcta* mating tests using male exposure to 10% basil oil revealed no positive effects on mating performance.

For *B. zonata*, the sexual maturation rate of laboratory flies has been determined. The influences of different doses of ME on mating competitiveness were investigated under laboratory conditions. The influence of age and duration of exposure to ME on male mating performance was studied under laboratory and field cage conditions. Male exposure to ME positively affected mating performance.

For *B. cucurbitae*, exposure to both cuelure (CUE) and raspberry ketone did not enhance male mating performance. A preliminary trial in Reunion on the effect of zingerone on melon fly mating performance had detrimental results. However, in Hawaii, CUE, melolure and zingerone improved male mating performance.

For *B. dorsalis* and *B. correcta* field cage experiments using commercial ME demonstrated positive effects of male exposure. The interaction between feeding on ME and post-teneral adult diet showed that exposure of sugar-only-fed males to ME increased their mating performance, while exposure to ME of sugar/protein-fed males increased their mating performance even further. Field cage tests comparing synthetic vs natural sources of ME (10% basil oil) have shown positive effects for synthetic ME, but not for the natural compounds from basil. For both species, ME treated sterile males are currently being released in operational SIT programmes in Thailand. In other experiments it was found that ME exposure did not affect dispersal or survival of sterile flies of both species in a mango orchard. Also, in other experiments, male exposure to both ME and 10% basil oil was found to increase longevity in a stress test.

For *B. tryoni*, field cage studies with older mature flies have shown that males exposed to CUE increased their mating competitiveness; however, young males failed to respond to CUE, orange oil (OO) and GRO. Other tests revealed positive effects on mating performance of males exposed to raspberry ketone.

3.3. Determination of optimal dose of ME/CUE that does not kill Bactrocera spp. male flies and increases their sexual performance.

For *B. philippinensis*, basic research was conducted on rates of sexual maturation and mortality of wild and laboratory reared males. The optimal feeding age and duration of exposure, and repeated feeding to ME have been determined in field cage mating studies. The relationship of fly age with ME feeding and the peak periods of ME daily feeding have been determined. Also, the diurnal pattern of ME feeding for wild *B. philippinensis* was determined.

3.4. Determination of optimal feeding age, and time and duration of exposure, as well as the interactions between feeding on ME/CUE, hormone treatment and/or nutrition in terms of Bactrocera spp. male competitiveness.

For *B. dorsalis* and *B. cucurbitae*, small cage and medium-scale open field SIT studies were conducted to measure induced sterility and male competitiveness. These studies indicated
high competitiveness for treated sterile males compared to wild males. Medium-scale, cost-effective methods of exposing males to ME together with assessment of longevity and field dispersal have been conducted for B. dorsalis. Small scale exposure of CUE to B. cucurbitae resulted in no effect on improving male mating performance. The interactions among feeding on ME/CUE, hormone treatment, and nutrition on improving male competitiveness have been studied in field cage tests. The effect of ME treatment on enhancing egg sterility levels under natural conditions in large field cages showed positive effects. For B. cucurbitae the effects of zingerone, melolure and cuelure on male sexual maturation and sexual success have indicated positive effect of all three compounds.

3.5. Study of the behavioural, physiological and ecological effects of Ceratitis spp. exposure to semiochemical supplements.

Exposure to olive fruits and GRO increased male longevity compared to non-exposed males. Exposure to orange oil (OO) and its components increased the sexual signalling of males and this effect is more pronounced in protein-fed flies. Exposure of protein-fed males to OO reduced life span. However, exposure of sugar-fed males to OO increased their life span. Other studies have shown that exposure of protein-fed males to GRO reduced their survival in laboratory tests. Behavioural observations revealed that GRO-exposed males exhibit higher rates of sexual signalling compared to unexposed males. Nonetheless, field dispersion and survival of GRO-exposed sterile male rates were similar to unexposed males in studies conducted both in Brazil, Hawaii, Australia, and Spain.

The remating frequency of females mated to sterile males treated with 0.1 ml/m$^3$ GRO was similar to those mated with wild males and lower than that for females mated with sterile unexposed males. GRO-exposed sterile males exhibit during sexual courting the same wing beat duration as wild males, and this was shorter than for unexposed sterile males.

3.6. Identification of chemical composition, including active compounds involved in Ceratitis spp., their modes of action, and comparison of their effects.

The effect of seven major components of OO was determined. Positive results have been obtained by limonene, b-myrcene, and linalool. A mixture of limonene, b-myrcene, linalool, a-pinene, geraniol (1:1:1:1:1) has been proven to be very effective for both wild and sterile males. Effects of both the mixture and OO were positive in protein/sugar-fed males but not in sugar-only-fed males. The optimum doses of exposure were determined for those compounds showing positive effects on increasing male performance. Positive effects of both OO and the mixture were found on protein/sugar-fed flies, but not on sugar-only-fed ones.

3.7. Comparisons of different semiochemical sources (both natural and commercial) in terms of their effect on Ceratitis spp. mating performance.

The effect of exposure of C. capitata males to fruits of five citrus species revealed that sweet oranges conferred the highest increase in male mating performance. Experiments using different varieties of sweet oranges revealed differential effects. Exposure of males to commercial citrus oils of the above species and varieties were found to be similarly effective. Other studies have shown that males responded significantly better to half ripe olive fruits compared to both green and fully ripe fruits. Sexually mature males respond significantly more than immature males. There was no difference in response to wounded and not wounded fruits in all fruit maturation stages.
For *C. rosa* the attractiveness of laboratory males to GRO and OO has been investigated. Sugar-only-fed males exposed to GRO and OO increase their mating performance in field cages. However, only GRO increased mating performance of protein/sugar-fed males.

### 3.8. Identification of an optimal delivery system and protocols to make these substances available in Ceratitis spp. SIT programmes.

The optimum exposure dose has been determined for OO and some of its major components in laboratory tests. Incorporation of the OO has been studied in both protein-sugar and sugar-only adult diets. Different oil proportions have been tested. The results revealed positive effects of OO in protein-sugar diet but no effects on sugar-only diet. Mating tests were completed in small field cages for OO exposed and non-exposed males with positive effects for OO exposed males.

Incorporation of GRO into adult diet has been studied. Some doses of GRO increased both male longevity and mating performance in field cages. Large-scale field studies involving ground releases have been conducted with GRO-treated males in Hawaii. A GRO delivery system was tested involving large rooms. The cost-effective GRO exposure to sterile males has been established and is now operational for SIT programmes in U.S.A, Guatemala, Australia, Israel, Spain, Portugal, and Brazil.

### 3.9. Assessment of the cost-benefits of incorporating these supplements into Ceratitis spp. SIT operations.

The benefits of GRO exposure can be realized at a very low cost, as revealed by cost-benefit analyses in SIT operational programmes in Australia, Brazil, USA, and Guatemala.

### 4. ABIOTIC ENVIRONMENT / RELEASE METHODS

#### 4.1. Compilation of all current procedures to ship, emerge, feed, hold, knockdown and release sterile flies.

Current procedures in different parts of the world to ship, emerge, feed, hold, chill, and release sterile flies were compiled and published under FAO in 2007 in an illustrated manual entitled “Guidance for packing, shipping, holding and release of sterile flies in area-wide fruit fly control programmes”.

#### 4.2. Assessment of the effect of varying relevant environmental parameters, such as temperature, relative humidity, and illumination on sterile male performance.

There has been progress on assessing the effects on sterile male performance of illumination and temperature in *C. capitata*. The effects of temperature and humidity in containers during transportation, receiving points and/or release machines have been evaluated for *C. capitata* for PARC boxes, automated ground release and aerial release machines. Exposure to higher light intensities increases mating behaviour for *C. capitata*. New fly emergence systems were developed based on containers, towers and on horizontal mesh tubes for *C. capitata*, and temperature, humidity effects and light conditions were evaluated to optimize them.

#### 4.3. Comparison of the effects of different emergence systems, including varying the size (volume) of the containers and resulting fly density, on sterile male performance in the field.
Comparison and evaluation of different emergence systems, including densities, volumes and ventilation on the resulting sterile male performance in the field was carried out for *A. ludens* and *C. capitata*. These included PARC and PVC boxes, various tower systems, paper bags, and other local systems, and a new fly emergence system was developed based on horizontal mesh tubes. Spatial and economic parameters of an emergence facility were assessed as well as yield of flyers, survivorship, mating success and flight distance. Also several methods of water supply were tested. Based on extensive evaluations under the CRP, all the California *C. capitata* programme moved its emergence, feeding and holding operations from boxes to towers.

4.4. **Assessing the interaction of different holding conditions and hormonal, nutritional and/or semiochemical supplements, on sterile fly performance.**

The assessment of the interaction of different holding conditions and hormonal, nutritional and/or semiochemical supplements on sterile fly performance resulted in the routine application of GRO under large scale holding conditions in operational *C. capitata* programmes. Also large scale evaluations of use of hormones and protein in adult diet have been conducted and as a result their use is now routine under large scale holding conditions in *A. ludens* and *A. obliqua* SIT programmes. Laboratory results on some of the interactions of different holding conditions and hormonal, nutritional and/or semiochemical supplements on sterile fly performance are available, but need to be implemented and assessed in emergence/release facilities for other fruit flies.

Evaluations of the cost-effectiveness and operational use of protein in the adult diet, in conjunction with both hormonal and semiochemical supplements still needs to be conducted in fly emergence centres. The effects of holding temperature and humidity on the production of pheromone were completed for several *Anastrepha* species. Holding conditions in relation to fly age and sexual maturation were assessed in terms of quality control parameters and persistence of flies in the field, indicating that in *C. capitata* sexing strains the release of older males is preferable resulting in more flies reaching mating age in the field. Other species that have longer pre-copulatory period would benefit even more from longer pre-release holding periods.

4.5. **Comparison of males resulting from standard conditions with males preconditioned to specific field conditions, such as high or low temperature, light cycle, light intensity, or elevation.**

Preconditioning of sterile males to specific field conditions was assessed in *C. capitata* and *B. tryoni* for temperature. However, further research is required especially on temperature, light intensity, photoperiod and elevation to compare adult males held in standard conditions with males preconditioned to specific field conditions.

4.6. **Design of fly holding systems to optimize the engineering and automation to improve SIT efficiency through reduction of construction and operational costs in order to improve fly quality at release.**

A design for a fly holding facility that optimises ME feeding and metabolising and fly holding in space and time was completed for *B. dorsalis*. Preliminary tests on interactions of insects with the semiochemical feeding structure have been tested in a small scale prototype.
4.7. Assessment of the effects of cold knockdown for immobilizing the flies on their performance, particularly for cold-sensitive species, and investigating alternatives if required.

The effects of cold knockdown for immobilizing flies were assessed on their performance in *C. capitata* and *B. tryoni*. Data have also been collected for *A. ludens* and *A. obliqua*. For *C. capitata* cold knockdown has no impact on pheromone production and on mating performance. For *A. ludens* and *A. obliqua* chilling was found to affect pheromone production and the mating performance during the first three days after chilling; however, flies recovered thereafter. Ground and aerial releases results indicate that allowing temperatures to increase to 16°C within release machines while releasing flies reduced problems with humidity caused by water condensation on flies due to the difference in temperatures in release machine and outside environment; this needs to be further confirmed.

4.8. Assessing effects on fly performance of different sterile fly release systems, as well as the interaction with hormonal, nutritional and/or semiochemical supplements.

The effects on fly performance of different sterile fly release systems, and the interaction with hormonal, nutritional and/or semiochemical supplements was assessed for some aerial and ground release systems for *C. capitata* in terms of dispersal and recapture. In addition, hormonally and nutritionally-treated *A. ludens* and *A. obliqua* released by air were evaluated.

**(b) OTHERS:**

- Enhanced collaboration among European, African, South American, North American, Asian, and Australian fruit fly laboratories and operational control programmes applying the SIT.
- Enhanced networking and information flow among fruit fly researchers, plant protection officers, and managers and personnel from a majority of operational fruit flies programmes from around the world.
- Capacity building for improved research and implementation in Africa, Asia, Australia, Europe and the Americas.
- Facilitation of the involvement of many students and young professionals in CRP activities, resulting in a significant number of Masters and PhD degrees.
- Transfer of technology from small scale research to field validation, to large scale application in SIT programmes.
- Dissemination of basic and applied findings on fruit flies in national and international professional meetings and in the scientific literature.
- Development of procedures and products that can be utilized by other entomological disciplines.
- Focused international attention on contribution of insect hormones and nutrition on reproductive development of insects.
- Led to research studies on the impact of hormones on sexual maturity in economically important insects including the honey bee, migratory and desert locust and *Varroa* mite.
- Induced graduate students to pursue research in areas of hormonal control of sexual reproduction.
- Identified methyl farnesoate as a new physiologically important hormone in insects.
- Demonstrated that insect endocrinology and chemical ecology can be effectively used to control important insect pests.
Recruitment of new workers in fruit fly control programme to implement use of semiochemicals.

New and improved ground and aerial release machines for insect release.

EFFECTIVENESS OF CRP:

(a) In reaching Specific Objective 1: For selected *Anastrepha*, *Bactrocera*; *Ceratitis* spp. assess the effects of hormone/analogue supplements, and develop hormone supplements procedures for incorporation into pre-release protocols for ongoing *Anastrepha* spp. SIT programmes.

Very significant progress has been made in evaluating and developing the use of hormone therapy to accelerate reproductive development for tephritid flies. The research documented that in many, but not all species, sterile males become sexually mature significantly earlier when hormone therapy is included in pre-release holding protocols but that optimum effect is only achieved when hormone treatment is coupled with a pre-release diet containing also a protein source. Additionally, females do not respond to hormone therapy to the same degree as males. Thus, irradiated males become sexually mature before females and are more likely to mate with wild flies. This advantage is particularly important for those species for which no genetic sexing strains are available. Coupling hormone therapy with an adult diet containing protein has been documented to accelerate sexual maturity in sterile males of *Anastrepha fraterculus*, *A. ludens*, *A. obliqua*, *A. suspensa*, *Bactrocera cucurbitae* and *B. tryoni* but does not affect males of the Mediterranean fruit fly or the ME consuming flies of the *B. dorsalis* species complex. However, alternative methods for improving sexual performance in these flies have been developed using semiochemicals in this CRP.

In addition, considerable progress has been made in developing delivery systems for large scale operational programmes. The most effective of these techniques is a sugar and protein paste containing 0.0015% methoprene which is painted on paper and provided to flies held in pre-release holding containers. This formulation allows for minimum use of diet, is easily prepared and is easily disposed of after use. The efficacy of this delivery system has been assessed and it is currently used in operational programmes for control of *A. ludens* and *A. obliqua* in Mexico at a cost of $15.00 USD/million flies. Concerns of environmental impact of disposal of large amounts of methoprene in spent diet have also been addressed and it has been determined that sodium hypochlorate (bleach) effectively deactivates methoprene. Overall this component of the CRP has been highly successful. A similar approach will allow extension of the technology to operational programmes for other species including *A. fraterculus*, *A. suspensa*, *B. cucurbitae* and *B. tyroni* for which the therapeutic techniques have been shown to be effective in laboratory and for most in field cage studies.

In reaching Specific Objective 2: For selected *Anastrepha* spp., *Bactrocera* spp. and *Ceratitis* spp. conduct basic research on adult diets and their effect on sexual performance of wild and sterile males.

At the inception of the CRP most SIT programmes used only sugar as the standard pre-release diet. In light of the research results from this CRP, sterile male performance has been enhanced in several programmes by adding a source of protein to the pre-release diet. Specifically, this is done for several species of *Bactrocera and Anastrepha*, in the Americas, Africa and Asia. The positive effect of protein nutrition on the pheromone, mating, latency period and decreased female remating has been amply demonstrated. The implementation requires relatively simple technology transfer.
Synergy between nutrition and hormonal supplements has been demonstrated for *Bactrocera* and *Anastrepha*. The ability of hormones to act effectively depends on the nutritional status of the male. Thus, adequate nutrition is the prerequisite to successful hormonal enhancement of male sexual performance.

The importance of bacteria as agents contributing to male fitness has been established, and various possibilities of manipulating them to further the goals of SIT operations have emerged.

**In reaching Specific Objective 3: For selected *Anastrepha, Bactrocera; Ceratitis* spp. assess the effects of semiochemical supplements, including natural sources, and develop delivery systems for exposure of methyl-eugenol (ME), and essential oils and other natural products to sterile males.**

The results have identified several compounds that improve sterile male performance in the field. These chemicals include (a) ME, and basil oils which improve the mating competitiveness of several *Bactrocera* species, including *B. dorsalis*, *B. philippinensis*, *B. zonata*, and *B. correcta*; (b) CUE, which enhances the performance of *B. cucurbitae*, and *B. tryoni* males; (c) raspberry ketone and/or zingerone which enhance the performance of *B. cucurbitae* and *B. tryoni* males; (d) GRO, α-copaene, or orange and other citrus oils, which enhance male performance of *Ceratitis capitata*, *C. rosa*, and potentially *Anastrepha fraterculus*; and (e) citrus and guava fruit volatiles, which enhance the mating performance of *male A. ludens* and *A. fraterculus*.

Methodologies for exposing large numbers of *C. capitata* males through aroma-therapy in large containers have been developed and are being implemented in ongoing SIT programmes in U.S.A, Mexico, Guatemala, Australia, Israel, Spain, Portugal, and Brazil. Millions of males are being routinely exposed to the aroma from a well defined dose of GRO in a very cost-effective manner resulting in a significant improvement in sterile male mating performance. This has been accomplished without any deleterious effect of GRO on male dispersal and survival in the field. Methods have been developed to effectively deliver ME to large numbers of sterile males in an ongoing SIT programme in Thailand for *B. dorsalis*, and *B. correcta*.

In addition, significant progress has been made in developing effective delivery systems for citrus oil to expose large number of *C. capitata* males including aroma-therapy and incorporation into the adult diet.

**In reaching Specific Objective 4: For selected *Anastrepha, Bactrocera; Ceratitis* spp. manipulate fly holding conditions at the fly emergence, holding and release centres to significantly improve male performance in the field, and assess fly release conditions (e.g. holding temperature, fly immobilization, fly density, fly handling and release system) for effects on male performance.**

In terms of improved pre-release environment, the effects of the relevant environmental parameters during fly holding conditions, such as temperature, relative humidity, and illumination, were assessed for sterile male performance in *C. capitata* and some *Anastrepha* and *Bactrocera* species. Also the effects of different systems to emerge and hold flies, including varying the volume of the containers and resulting fly density, were compared in terms of sterile male performance in the field *C. capitata*. The interaction of different fly holding conditions and hormonal, nutritional and / or semiochemical supplements, on sterile fly performance were assessed for *A. ludens*, *A. obliqua* and *C. capitata*. Automated semiochemical feeding system and structure was designed and a small scale prototype was built and tested for *B. dorsalis* to deliver ME.
In terms of effects of release conditions the effects of chilling and cold knockdown for immobilizing the flies for release on their performance were assessed for *A. ludens*, *A. obliqua* and *C. capitata*. The effects on fly performance of some sterile fly release systems and some interaction with hormonal, nutritional and/or semiochemical supplements was assessed for *A. ludens*, *A. obliqua* and *C. capitata*, allowing to improve release systems.

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

The Agency project overall objective is to assist Member States in achieving sustainable fruit and vegetable production and in facilitating trade by applying area-wide integrated SIT suppression/eradication programmes against fruit flies of economic importance. The CRP contributed significantly to this objective by reducing the cost and increasing the effectiveness of fruit fly SIT programmes through improved performance of mass produced sterile males in operational fruit fly SIT programmes, specifically through manipulations implemented at the emergence and release facilities. The efficiency of the implementation of SIT for tephritid fruit flies was enhanced by a much better understanding of the hormonal mechanism and interaction between nutrition and hormones that regulate sexual maturation of males. Supplementation of the diet offered to sterile males of various species with nitrogen rich compounds also contributes significantly to this objective. Post-teneral exposure of sterile males to semiochemicals significantly improves male mating performance in the field, thereby increasing the cost efficiency of the SIT. The same applies to improved large scale fly holding and release systems.

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

Some problems arose with the movement of some CRP members to the different countries where the RCM's were held, due to visa and immigration issues.

While several action programmes have been responsive, and have readily accepted and implemented the cost effective hormonal, nutritional or semiochemical treatments, some other programmes have been slow to adopt and implement the findings of the CRP.

The diversity of pest species included in the research, together with the different modes of action of the active compounds, have made it difficult to apply common protocols to all species. Transferring results from the laboratory to mass exposure of sterile males for certain pest species and supplements has been difficult and will require in some of these cases further research to be able to move to large scale application.

Impact of the CRP:

The CRP demonstrated that hormone therapy coupled with feeding protein to adults significantly accelerates male maturation and sexual performance among *Anastrepha* species and some *Bactrocera* species with no negative side effects (survival and competitiveness). Additionally, because females do not become mature as rapidly as males after hormone therapy sterile males can mate with wild females early in their lives rather than mating with sterile females. These factors result in significant cost savings associated with holding males prior to release in SIT programmes and fewer males die before mating with wild females. The validation in a large number of pest fruit flies of these findings has resulted in the incorporation of this major breakthrough into large scale action programmes against several pest species. For several other pest species, laboratory and semi-field tests strongly indicate that application of nutritional and/or hormonal pre-release treatments will also produce an increase in SIT efficiency, and thus can also be adopted by programme managers.
The development of cost-effective semiochemical treatments that improve sterile male mating performance has been highly desirable. Implementation of such feasible treatments has significantly increased the effectiveness of SIT programmes for several fruit fly pest species, leading to reduced chemical applications to the environment, and increased food production and quality. Improved fly emergence, holding and release procedures, and the compilation of all this knowledge into a manual, have benefited all operational fruit fly SIT programmes. Knowledge gained and practical procedures developed during this CRP are transferable, at least in part, to other insect pest species with control programmes that include an SIT component.

Relevance of the CRP:

The CRP was extremely relevant and well designed by a group of external consultants to address the needs of fruit fly SIT programmes. Focusing research on the post-factory factors that affect sterile male performance was very relevant to maximize the effectiveness of the sterilized insects that have been mass produced. The CRP succeeded in focusing the attention of fruit fly researchers and action programme managers on the importance of this post-teneral period. Effectively the CRP brought these two groups together, resulting in goal-oriented applied research that produced tangible results. Dissemination of these results, either as discrete operational protocols (that are already being applied), or in the peer reviewed scientific literature, provides universal access to the advances made. In addition, the approaches and methods developed in this CRP have inter-specific implications. They can be adopted by programmes dealing with pests or vectors other than tephritids (e.g., Lepidoptera, culicid mosquitoes and tsetse flies).

Recommended Future Action by Agency:

- Publish results of CRP in a special issue of the peer-reviewed international scientific “Journal of Applied Entomology”.
- Update the SIT fly handling and release manual to include the protocols developed during this CRP.
- Facilitate the adoption of protocols of pre-release feeding in additional fruit fly action programmes.
- Pursue novel approaches to further improve fruit fly SIT operations. In particular, future CRP's could deal with one or more of the following: The role of microorganisms in tephritid biology. The relationship between larval and adult nutrition, and their effect on adult fitness and sterile fly performance. Naturally occurring compounds that affect male sexual biology, and the mechanisms involved.
- Support further studies on the biological basis of the synergetic effects of nutrition, hormones and semiochemicals on male sexual performance, and the mechanisms whereby hormones and semiochemicals affect male sexual performance.
- Support research on the interaction of hormones and semiochemicals from plants and bacterial sources on the regulation of reproductive development in tephritid flies.
- Continue studies of the effects of hormone and diet supplementation on other pest species, and extend technology currently developed for action programmes to the other species studied.
- Identify the main compounds in guava and/or other fruit species that improve mating performance of Anastrepha fraterculus, A. obliqua, and A. ludens is needed.
- Determine optimal doses of the identified compounds and modes of exposure to fruit flies.
• Carry out additional research to detect natural sources of ME, and dose effectiveness should be addressed in large scale SIT programmes for Bactrocera spp.
• Study integration of ME to pre-release adult diet for additional Bactrocera species.
• Ascertain optimal fly densities in different types of holding containers and systems.
• Support further development of semiochemical mass feeding/administering systems to simulate real SIT large scale situations.
• Investigate delivery systems that reduce fly damage and mortality caused by less than optimal delivery systems in current use, and compare screw augers, conveyor belts and other mechanisms.
• Support more work on the development of ground release machines and systems for different situations, e.g. towns, peri-urban areas, farm gardens/backyards, orchards and mountainous/other difficult terrain. Systems need to be developed for different capacities of release machines depending on programme requirements.
• Support further work in aerial release systems to reduce cost and wider application.
• Develop and assess unmanned aerial systems with capacities tailored to specific programme needs and scales to bring significant cost savings.
• Support further investigations on the conditions affecting sterile flies in aerial and ground release machines.

Resulting Publications:


Publications in Press, Submitted and in Preparation:


Barnes, B. Improving the environmental conditions for sterile Mediterranean fruit flies in holding containers prior to release. Journal of Applied Entomology.


Orankanok, W., S. Chinvinikul, A. Sawatwangkhoung, S. Pinkaw and S. Orankanok. Comparison of young sterile male exposed to commercial methyl-eugenol and basil oil on mating competitiveness. Journal of Applied Entomology.


Weldon, C.W., J. Prenter, and P.W. Taylor. Activity patterns of Queensland fruit flies (Bactrocera tryoni) are affected by both mass rearing and sterilization. Physiological Entomology. (in press)