**1. CRP Title**

*Evaluating the Use of Nuclear Techniques for the Colonization and Production of Natural Enemies of Agricultural Insect Pests*

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

**3. Project Officer**: Jorge Hendrichs

**4. Period Covered:** 1999-2005

**5. List of Participants**

| Name (CSI) | Institute/Country |
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**Research Co-ordination Meetings**

| Meeting | Date | Location |
|---|---|---|
| 1st RCM | 22-26 October-1999 | Vienna, Austria |
| 2nd RCM | 18-22 June 2001 | Tapachula, Mexico |
| 3rd RCM | 3-7 November 2003 | Vienna, Austria |
| 4th RCM | 13-17 May 2005 | Vienna, Austria |

## 6. Objectives of the CRP

*Overall:*

To increase the cost-effectiveness, trade and safety in the use of biological control agents of insect pests of agriculture.

*Specific:*

To assess the potential roles nuclear techniques can play in improving production, and facilitating trade and use of quality biological control agents in integrated pest management.

## 7. Outputs

| **Expected** | Final Status |
|---|---|
| 1. Improvements in rearing media/host and utilisation of by-products of mass rearing facilities. | 1. Procedures developed for production of three biological control agents using irradiation. |

| | |
|---|---|
| 2. Some shipping-related problems overcome. | 2. Procedures developed for facilitating shipping of two natural enemies using irradiation. |
| 3. Use of sub-sterile or sterile hosts or prey in the field as supplemental food to natural enemies. | 3. Procedures assessed for using in the field sterile hosts of two insect pests to supplement natural enemies. |
| 4. Integration of biological control with SIT or inherited sterility. | 4. Procedures assessed for integrating in the field SIT/inherited sterility and biological control for two insect pests. |
| 5. Demonstrated potential for using reproductively inactivated exotic agents for confirmation of host specificity of potential biological control agents. | 5. Procedures under development for evaluating under natural conditions two exotic biological control agents for confirmation of host specificity. |

**RESEARCH**:

The wide range of research performed during the CRP was aimed at exploring the potential of using nuclear techniques in facilitating the various applications of biological control agents against insect pests in agriculture. This was the first CRP in this area, which is largely **independent** of the Sterile Insect Technique.

Specific advances in the four main research areas of the CRP:

## A. REARING

*A1. Suppression of Host Immune Reactions*

**The Problem:**

Host immune reactions may reduce rearing efficiency or prevent the use of factitious or non-habitual hosts that are easier or more economical to rear.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

Exposure to radiation has been shown to suppress host immune system responses. This may make irradiated larvae of older instars more suitable for parasitoid development and thus increase rearing efficiency and parasitoid quality.

**Achievements:**

The encapsulation of Sephadex beads in hemolymph of *Galleria mellonella* in-vivo and in-vitro demonstrated that this process involves cellular mode of capsule formation. Irradiation of *G. mellonella* larvae leads to incomplete or no encapsulation of Sephadex beads (in vivo and in vitro). These effects could be attributed to damage of hemocytes by irradiation. *G. mellonella* larvae of 3$^{rd}$ and 4$^{th}$ instars irradiated with dosage of 65 Gy were found to be suitable for parasitisation by *V. canescens*. Overall, the results show that irradiation of *G. mellonella* larvae with gamma rays may convert this insect from non-habitual to habitual host of the endoparasitoid *V. canescens.*

A dose of 60-80 Gy suppressed the immune reaction in *Chilo infuscatellus*, allowing 4$^{th}$ and 5$^{th}$ instar larvae to be parasitised by *Cotesia flavipes*.

In insects, incidental entry of microbes or parasitoids elicits a host defence response, which mainly consists of phenoloxidase (PO) pathway. PO participates in sclerotisation, melanisation, and related immune reactions to foreign organisms. The invasion of foreign organisms is recognized by a specific protein, which activates PO pathway consisting of prophenoloxidase (PPO) and PPO activating enzyme. Analysis of the components of insect immunity in response to radiation is in progress to help develop effective biological control strategies.

*A2. Extension of Storage Time for Hosts or Prey*

**The Problem:**

Normal host/prey development limits storage of host/prey material.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

Use of radiation may be used to arrest insect development and thus facilitate the development of procedures that would allow for storage and stockpiling of hosts or prey.

**Achievements:**

Studies on *Ephestia kuehniella, Spodoptera litura, Sitotroga cerealella*, and *Musca domestica* showed that irradiation caused a prolongation in the development of host stages suitable for parasitization, thus facilitating the use of these hosts under mass rearing conditions. Doses between 75 and 150 Gy used to inhibit potato sprouting can also increase the incubation period of potato tuber moth *P. opercullela*.

Host eggs of *E. kuehniella* irradiated at 200 Gy could be stored at 4°C for up to 30 days without any loss in parasitoid production, both number and quality of *Trichogramma evanescens* and for up to 60 days with minor decrease compared to controls without storage. Parasitoids in diapause could be stored in irradiated (200 Gy) host eggs for a period of 50 days without adverse effect on emergence. Acceptance of irradiated eggs was not different to nonirradiated eggs.

Irradiation of C. *infuscatellus* host larvae parasitized with *Cotesia flavipes* in conjunction with low temperature (10°C) could prolong the storage of parasitoid.

*E. kuehniella* larvae that were irradiated with 200 Gy and parasitized by *Venturia canescens* could be stored at 4°C for 4 weeks to allow parasitoid emergence. However, longevity and fecundity of parasitoids decreased with storage time.

*A3. Extension / Enhancement of Host Suitability*

**The Problem:**

Normal host development limits the time interval or host quality when a host is suitable for parasitization.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

Use of radiation may be used to delay normal insect development and thus extend the time interval or modify the internal host environment when a particular host stage is available for use by the parasitoid or help to regulate (slow-down, synchronize, or enhance) parasitoid development within the irradiated host.

**Achievements:**

Results have shown that radiation can be used to delay normal insect development and extend the time when a given host stage is suitable for use by parasitoids. Examples are related to the use of parasitoids in poultry production to control *Musca domestica*, to control *Anastrepha* spp. in mixed fruit orchards, and to control *Ephestia kuehniella* and *Plodia interpunctella* in mills and warehouses. In addition, radiation has been used to extend the time *Sitotroga cereallela* as factitious host is available for *Trichogramma chilonis* to control *Chilo infuscatellus* and two other sugarcane borer species, *Tryporhiza nivella* and *Emmalocerra depressella*.

Irradiation of *Anastrepha* spp. 3$^{rd}$ instars with a dose of 45 Gy allowed extending the parasitisation period and increasing under mass rearing conditions the quantity and quality of *D. longicaudata* produced. Irradiation of *B. carambolae* eggs with 30-50 Gy extended the larval period available for parasitization of *P. incisi*.

Irradiation of the uzi fly pupae with doses ranging from 0.5-8 Gy allowed extending the parasitization period and increased the progeny production of *Nesolynx thymus*. Dose range from 0.5 to 8 Gy in case of early pupae of factitious dipteran hosts and 70 to 90 Gy in case of late pupae could increase to progeny production of parasitoid, *N. thymus*.

UV-sterilization of the host eggs markedly increased the parasitization capacity of *T. chilonis* with respect to unsterilized host eggs of *Corcyra cephalonica* and *S. litura*, and further emergence of the parasitoid from UV-sterilized host was significantly enhanced.

Irradiation with 250 Gy to *H. armigera* eggs can prolong the period of parasitization by *T. chilonis.* The developmental time of $F_1$ larvae of *L. dispar* irradiated in the first instar was extended, thus extending the period available to the natural enemy complex.

The host suitability of *M. domestica* pupae could be extended up to 2 months, although the reduction in the host quality in elicitation of parasitization increased with time.

*A4. More Efficient Utilization of Sub-Products from SIT Mass-Rearing.*

**The Problem:**

Insect mass-rearing programmes often produce relatively large numbers of substandard material or have excess production of particular life stages that are discarded.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

Sub-products of insect mass-rearing programmes (e.g., excess or “off” season production or products that do not meet minimum quality standards) may be irradiated and used to support the production of natural enemies and thus improve the overall (cost) efficiency of an SIT mass-rearing system.

**Achievements:**

Research has confirmed that insect mass-rearing programmes often have excess production of particular life stages or produce significant amounts of substandard material that is discarded. These can be irradiated and used to support the production of natural enemies. Examples include the use of excess egg production in *Ceratitis capitata* and *Anastrepha ludens* mass-rearing facilities to produce egg parasitoids, as well as the use of remnant larvae and pupae to produce larval and pupal parasitoids.

*A5. Physiological and/or Behavioural Stimulation Effects of (Low) Doses of Radiation to Natural Enemies*

**The Problem:**

Field performance of laboratory-reared parasitoids and predators is a concern.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

Very low doses of radiation may stimulate a variety of physiological (e.g., pesticide tolerance) and behavioural (e.g., increased longevity and searching ability) processes in insects that may be beneficial. As such, very low doses of radiation may be useful in improving field performance of laboratory-reared parasitoids/predators.

**Achievements**:

Irradiation of adult *Habrobracon hebetor* with 10 Gy gamma radiation resulted in significant increase of adult longevity and oviposition period of the females ovipositing on *Galleria mellonella* larvae. Their increased fecundity was associated with extended oviposition period.

Irradiations of adult *Venturia canescens* (thelytokous strain) with doses of 5 and 10 Gy reduced their parasitism on *E. kuehniella* larvae, whereas treatments of the parasitoids with 1 and 2 Gy significantly increased the rate of parasitism.

In *Trichogramma chilonis* a low dose of radiation (100-200 mGy) altered the sex ratio in favour of females. Irradiation of adults *V. canescens* with 3 Gy significantly increased the rate of parasitism of *E. kuehniella* larvae.

When infective juveniles of the entomopathogenic nematode *Steinernema glaseri* were irradiated and bioassayed one week post-irradiation, faster host morbidity and mortality was observed at doses of 1.5 and 2.5 Gy (the effect being distinctly evident at 2.5 Gy). Increased harvesting of irradiated infective juveniles of *S. glaseri* at 2.5 Gy was also noticed when compared to the control.

A stimulatory dose of 200-250 mGy applied to *T. chilonis* parasitoids significantly increased the number of parasitized *H. armigera* eggs per *T. chilonis* female and the number of offspring produced.

Irradiation of adult *T. evanescens* with 5 Gy did not alter the rate of parasitization; higher doses caused adverse effects.

*A6. Use of Radiation as a Tool to Study Host-Natural Enemy Interactions*

**Problem:**

Behavioural and physiological interactions between host and parasitoids are complex, often difficult to study and not well understood.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

Nuclear techniques may be used to selectively modify certain physiological processes in the host (e.g., defence mechanisms, hormone metabolism) thereby facilitating the study of particular host-parasitoid interactions. Nuclear techniques may be used to modify or terminate certain parasitoid processes that affect host physiology and behaviour (e.g., sterilizing the parasitoid egg).

**Achievements:**

Irradiation of *Glyptapanteles liparidis* wasps (24, 48, and 96 Gy, respectively) caused temporary sterilization and a reduction in oviposition. Irradiation reduced total number of eggs laid per female but did not reduce longevity. Effects of "pseudoparasitization" of *Lymantria dispar* larvae by irradiated female wasps (i.e., injection of sterilized eggs together with polydnavirus and venom) indicate that this method can be used to study the influence of parasitoid associated factors. Pseudoparasitism of *L. dispar* larvae caused delayed larval development, morphological abnormities and high mortality during pupation or in the pupal stage. The immune response (hemocytic encapsulation and hemolymph melanization) of the host larva was suppressed by pseudoparasitization. The technique was used in experiments to separate effects of parasitoid larvae from associated factors in vitro and re-combine them by implanting them into new hosts. Pseudparasitization prevented encapsulation of implanted parasitoids; but only the complete recombination of larvae with both teratocytes and polydnavirus/venom allowed successful emergence of parasitoids. Separation and recombination of parasitoid and associated factors was used to study parasitoid-induced alterations of juvenile hormone metabolism of *L. dispar*. Pseudoparasitized larvae showed reduced activity of juvenile hormone esterase. Implantations of parasitoid larvae into pseudoparasitized hosts showed that parasitoid larvae release juvenile hormone into the host. Pseudoparasitization was used to study interaction of *L. dispar* host larvae with various entomopathogenic microsporidia. Pseudoparasitization slightly, but significantly, increased spore production of the studied microsporidia in the host. However, semi-permissive hosts did not become fully permissive.

## B. HANDLING-SHIPMENT-RELEASE AND TRADE

### *B1. Reproductive Sterilization of Host / Factitious Hosts / Prey to Improve Rearing*

**The Problem:**

Continued development and emergence of non-parasitised fertile hosts, factitious hosts, as well as of unused prey (pest) insects during rearing of natural enemies often require additional steps in handling, thereby decreasing the efficiency of rearing systems.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

Radiation can be used to reproductively sterilize hosts, factitious host or prey, thereby inhibiting further development and preventing the emergence of unused individuals. This application of nuclear techniques would:

- Reduce the handling procedures required during rearing of natural enemies, thereby increasing the cost effectiveness of the rearing process and the quality of the natural enemy product.
- Allow for the earlier shipping of hosts together with natural enemies without the need to wait for emergence of unused hosts.
- Facilitate the preservation of purity of host, prey and/or natural enemy strains.
- Provide a cleaner product for customers purchasing/using natural enemies produced in this fashion.

**Achievements:**

During rearing of natural enemies not the entire host material is parasitized or consumed, requiring additional steps in handling prior to shipment of the natural enemies to prevent the release of pest insects. Radiation now has been successfully used to irradiate host larvae and prevent adult emergence of Mediterranean flour moth *Ephestia kuehniella*, Indian meal moth *Plodia interpunctella*, sugarcane borer *Chilo infuscatellus*, Mediterranean fruit fly *Ceratitis capitata*, Mexican fruit fly

*Anastrepha ludens*, West Indian fruit fly *A. obliqua* and zapote fruit fly *A. serpentina* in order to rear their parasitoids. Radiation has also been used to irradiate eggs of *A. ludens*, and pupae of house fly *Musca domestica* and *A. ludens* to prevent adult emergence in the mass rearing of egg and pupal parasitoids for these pests. Releases of these parasitoids without the simultaneous release of pest insects have only been made possible by the application of radiation.

Host radiosterilization of common cutworm or tobacco caterpillar *Spodoptera litura*, at 40-70 Gy was used for the safe transport of entomopathogenic nematodes (EPNs). A reasonable degree of bioinfectivity was retained by EPNs carried within radiosterilized hosts. 40-70 Gy could be considered for inundative releases of EPNs, whereas 40 Gy was a feasible dose for inoculative releases.

*B2. Facilitating the Shipment of Natural Enemies and Host Material*

**The Problem:**

There exists a real or perceived risk that shipping natural enemies with host/prey material will lead to introduction of non-native, pesticide resistant or new strains of pest insects into new areas or countries. This may exacerbate the ever-stricter quarantine regulations required to obtain permits for natural enemy shipment.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

Use of radiation has the potential of:

a) Killing or reproductively sterilizing host/prey to provide the required quarantine security to overcome regulatory barriers, and thereby facilitating and encouraging national and international trade in biological control agents.
b) Reducing the risk of inadvertently shipping hitchhiking arthropods with host/prey.
c) Extending the period of suitability of host or prey (as food) during shipping.
d) Allowing the addition of safe, nutritional supplements (in the form of host/prey) to shipments of natural enemies that will improve/maintain their quality.
e) Allowing timelier delivery of natural enemies by eliminating the time required allowing non-parasitised hosts to emerge, and eliminating the need to separate emerged adult host from parasitised hosts.
f) Allowing the customers more flexibility in release timing of natural enemies.

**Achievements:**

Research on the use of pest mite *T. urticae* eggs to provision shipments of several species of predatory mites has confirmed that radiation at a dose of 280 Gy or less, depending on the age of the host eggs, can be used to eliminate the risk of introducing fertile pest mites, or other hitch-hiking arthropods, and at the same time allow the inclusion of nutritional supplements in the form of host material to maintain quality.

Irradiation of house fly pupae has proved very beneficial for the shipment of house fly pupal parasitoids by allowing for early shipment of just parasitised pupae while ensuring clean shipments to the customer because you do not have to wait for the emergence of unparasitised hosts.

The use of irradiated host material also makes it possible to ship fruit fly parasitoids to different countries without the risk of fly emergence. This can be important when the host used in the mass rearing is an important quarantine pest. For example, Mexico has now been able to send fruit fly parasitoids to South America reared on *A. ludens*. These applications of radiation will help facilitate and encourage the national and international trade of natural enemies.

*B3. Shipping of Sterilized Pests/Factitious Hosts or Prey (in the Absence of Natural Enemies*)

**The Problem:**

The same real or perceived risk exists of shipping fertile hosts or prey material, even in the absence of natural enemies, will lead to accidental introduction of non-native, pesticide resistant or different strains of pest insects into new areas or countries. This may be exacerbates by the ever-stricter quarantine regulations required to obtain permits for shipment of insects.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

The use of radiation will allow for the commercial shipment of sterilized host individuals, from one laboratory or insectary to another, both within and between countries, without the risk of introducing unwanted pest species or strains. Commercial laboratories will also be able to rear the same strain of natural enemy using the same host material, insuring a standardised quality of the product for their customer.

**Achievements:**

The commercial need to ship sterile host/prey material in the absence of natural enemies for use and redistribution by smaller rearing facilities and to standardize host material to insure product quality was successfully addressed. One example is the shipment of sterile house fly *Musca domestica* pupae to rear their parasitoids in other locations. Irradiation was shown to maintain the suitability of pupae for parasitisation over a longer period of time, which now makes it possible to store these pupae and ship them as needed between facilities for rearing house fly parasitoids.

## C. FIELD APPLICATION OF REPRODUCTIVELY STERILIZED HOSTS TO ENHANCE BIOLOGICAL CONTROL

*C1. Combination of Augmentative Releases: SIT/ $F_1$ Sterility and Natural Enemies*

**The Problem:**

Agricultural and forest production is adversely affected by many insect pests which have traditionally been controlled with a heavy emphasis on chemical pesticides. Resistance to many insecticides has been documented, leading to increased use rates, which in return can exacerbate the adverse affects of pesticides on the environment.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

Nuclear techniques (SIT/ $F_1$ sterility) and augmentative releases of natural enemies (parasitoids, predators, nematodes, and insect pathogens) can significantly reduce insect pests populations. Combining these tactics can yield both additive and synergistic effects. These combined augmentative releases would be compatible with traditional IPM programmes that could include resistant plant varieties, biopesticides / biorational methods, cultural practices, and mating disruption.

**Achievements:**

Nuclear techniques (SIT/$F_1$ sterility) and augmentative releases of natural enemies were shown to be compatible strategies that can yield both additive and synergistic effects. Laboratory studies and field trials with *Helicoverpa armigera*, *H. zea*, *Phthorimaea operculella, Lymantria dispar, Spodoptera litura, S. exigua,* and *Plutella xylostella* indicated that progeny from irradiated moths were acceptable as hosts for egg and larval parasitoids.

Experimental proof under laboratory conditions showed that $F_1$ sterility and *Trichogramma* releases are effective in suppressing *P. opercullela*. Because of its tolerance to higher temperature, *T. principium* was chosen to be released in combination with 250 Gy-irradiated moths to suppress *P. opercullela* populations. Two properly timed releases of *T. principium* and irradiated moths produced the greatest reduction in *P. opercullela* $F_3$ progeny, demonstrating the synergistic effects of combining SIT/$F_1$ sterility with egg parasitoids.

In addition, *L. dispar* larvae that were reproductively sterilized by irradiation were found to be suitable carriers for the transmission of nuclear polyhedrosis virus to field pests populations.

Eggs laid by false codling moths (FCM) treated with 150-200 Gy were acceptable and suitable for development of the egg parasitoid *Trichogrammatoidea cryptophlebiae* under laboratory conditions. Field-cage evaluations in citrus orchards in South Africa revealed that releases of irradiated (150 and 200 Gy) FCM combined with releases of *T. cryptophlebiae* provided synergistic control of FCM populations.

The parasitization rate by *T. chilonis* parasitoids was significantly higher in *H. armigera* eggs resulting from crosses of sterile and normal, or sterile and sterile moths, than those from unirradiated moths.

The compatibility of the application of entomopathogenic nematodes (EPNs) with $F_1$ sterility has been demonstrated towards population suppression of *Spodoptera litura* in case of high pest density, relatively low density and quarantine. Various feasible modes of integration of these two biorational strategies are being considered. Furthermore, the feasibility of augmentative releases of EPNs within radio-sterilized hosts in conjunction with the use of biorational pesticides and moulting hormone agonist has been demonstrated.

### *C2. Supplement Hosts for Natural Enemies Prior to Pest Population Outbreak*

**The Problem:**

Many insect pests have demonstrated cyclic population outbreaks. Although these outbreaks may be predicted, effective and environmentally friendly control strategies are needed to reduce the effects of these economically damaging events.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

An increase in the number of host insects available for natural enemies can increase the population density of natural enemies before a cyclic pest outbreak begins. In this way, an optimum level of natural enemies can be available to prevent or at least reduce the level of expected pest outbreaks. Nuclear technique can be used to produce sterile host insects or host insects with inherited sterility ($F_1$). Releasing sterile insects as hosts for the natural enemies can increase the number of host insects available for natural enemies without increasing the risk that the released insect pest will cause economic damage in the future.

**Achievements:**

Doses of radiation required to reproductively sterilize pest eggs and larvae of gypsy moth *Lymantria dispar* were determined. Irradiated gypsy moth eggs, as well as $F_1$ eggs and larvae resulting from irradiated parents, were studied in the field and found to be acceptable and suitable as host for a number of natural enemy species. Parasitoids did not differentiate between $F_1$ and untreated larvae.

*C3. Supplemental Hosts for Seasonal Maintenance of Natural Enemies*

**The Problem:**

Pest populations can vary greatly from generation to generation. During periods of low pest densities, population levels of their natural enemies are sometimes reduced to very low levels. The low population levels of natural enemies are then unable to effectively respond in a timely manner to rapid build-ups in the pest population.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

The use of SIT or $F_1$ sterility could provide supplemental hosts in the form of sterile eggs, larvae, and pupae. These supplemental hosts could sustain higher population levels of natural enemies during critical periods so that future pest population increases would be moderated. An example of this application would be the release of an egg parasitoid during a control programme integrating the SIT so that sterile eggs deposited by irradiated insects could be used by the parasitoid.

**Achievements:**

During periods of low pest densities, population levels of natural enemies can be very low and unable to respond immediately to an increase in the pest population. Irradiated *Helicoverpa armigera* and *Plutella xylostella* moths released in the field during critical periods laid eggs that served as host for feral egg parasitoids and caused the parasitoid population to increase.

The provision of supplemental hosts (irradiated/sterile host eggs) to *Trichogramma chilonis* early in the season to sugarcane fields allowed to build-up populations, or enhanced the survival of the parasitoids during critical periods thereafter. This approach is currently providing effective management of several species of sugarcane borers in 1,090 ha of sugarcane in Pakistan.

Eggs laid by false codling moths (FCM) treated with 150-200 Gy were acceptable and suitable for development of the egg parasitoid *Trichogrammatoidea cryptophlebiae* under laboratory conditions. Field-cage evaluations in citrus orchards in South Africa revealed that releases of irradiated (150 and 200 Gy) FCM combined with releases of *T. cryptophlebiae* provided synergistic control of FCM populations. A pilot study in 35 ha of citrus planned for October 2005 will examine the effect of releasing irradiated FCM to control FCM populations and to increase the population of *T. cryptophlebiae*.

After the introduction of *V. canescens* parasitoids into a mill and a dried fruit store the number of collected *E. kuehniella* moths decreased by ca. 80 % in comparison to controls.

*C4. SIT Against Natural Enemy Pest*

**The Problem:**

In certain cases where an insect provides a useful service, natural enemies of the useful insect are considered to be a pest. Examples of this relationship would include parasitoids of the silkworm, *Bombyx mori*, Varroa mites that attack honeybees, or natural enemies of weed herbivores.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

In this type of relationship, the natural enemies could be sterilized with nuclear techniques and released as part of programme that includes an SIT component to reduce the detrimental effects of the natural enemies on the useful insect.

**Achievements:**

Radiation biology studies were conducted on the uzi fly, *E. sorbillans*, a tachinid parasitoid of *Bombyx mori*, to determine the optimum dose required to sterilize this natural enemy pest. A dose of 70-90 Gy applied to late pupae resulted in complete sterility of adult uzi fly females and males. Mating competitiveness studies showed that sterile males could effectively compete with wild males for wild females.

## D. FACILITATION OF CLASSICAL BIOLOGICAL CONTROL AND NATURAL ENEMY MONITORING

*D1. Use of Reproductively Inactivated Agents for Final Confirmation of Host Specificity of Potential Exotic Biological Control Agents*

**The Problem:**

The importation of exotic natural enemies, particularly insect herbivores of plant pests, is becoming increasingly difficult due to concerns over the possibility that imported natural enemies may shift and become pests of beneficial or protected species. In some cases, despite extensive and positive pre-release studies under quarantine conditions, the release of promising biological control agents is ultimately rejected because of remaining doubts about their host specificity.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

Radiation may be used to reproductively inactivate exotic natural enemies so that they can be released and studied under actual field conditions without the risk of establishing breeding populations. The use of reproductively inactivated forms would allow further assessing and confirming oviposition behaviours and host (acceptability) associations. The use of $F_1$ sterile larvae of herbivores, being considered for release against plant pests, also would allow field-testing larval feeding preferences and the ability of these larvae to develop and survive on related plant weeds that are of concern.

**Achievements:**

A model system including *Opuntia* spp. and the cactus moth (*Cactoblastis cactorum*) has been developed to study the host range of an exotic herbivore. Radiation biology studies revealed that the optimum dose at which females are sterilized and males remain partially fertile and produce sterile progeny is 200 Gy. Whole plant and single cladode host preference tests demonstrated that *C. cactorum* females mated with males irradiated at 200 Gy exhibit normal oviposition preference and can be used safely under field conditions to predict host range.

Another system under evaluation involves the exotic herbivore *Episimus utilis* Zimmermann (Lepidoptera: Pyralidae) for the biological control of Brazilian pepper tree. *E. utilis* is currently in quarantine in Florida.

*D2. Use of Sterilized Hosts for Exploration of New Natural Enemies and for Monitoring Natural Enemy Field Populations*

**Problem:**

The collection of new exotic natural enemies or the monitoring of field populations of native natural enemies is sometimes complicated by the fact that hosts are rare or difficult to locate.

**Identified Nuclear Techniques that Can Help to Address the Problem:**

Reproductively inactivated host insects may be placed in the field in strategic locations as sentinels to aid in the exploration and collection of new natural enemies. These sentinels may also be used to monitor natural enemy populations. Furthermore, the use of live but reproductively inactivated hosts will eliminate the risk of increasing pest populations.

**Achievements:**

Monitoring of field populations of natural enemies is sometimes complicated by the fact that hosts are rare or difficult to locate. Radiation biology studies were conducted to determine appropriate doses to reproductively inactivate certain life stages. Eggs of *Sitotroga cerealella*, a fictitious host of *Trichogramma* irradiated at 25 Gy were used to monitor the establishment of released *Trichogramma* in sugarcane fields.

*Lymantria dispar* larvae resulting from male parents irradiated at 30 and 120 Gy were used in forests for monitoring the number and type of parasitoids and pathogens.

Reproductively inactivated larvae (400 and 600 Gy) of *Ephestia kuehniella* and *Plodia interpunctella* were placed in warehouses and mills to monitor the number of *V. canescens* and *H. hebetor* throughout the season.

Sterilized larvae of *Anastrepha* spp. and *Bactrocera* spp. fruit flies were used to monitor populations of natural enemies.

The doses to reproductively inactivate *Cactoblastis cactorum* have been determined. Experiments are planned to determine whether sterile egg sticks from irradiated adults can be used to survey for generalist egg parasitoids occurring in infested areas.

Sterilized *Musca domestica* pupae in traps have also been used for exploration of wild populations of Pteromalid parasitoids in the field and under conditions of livestock productions.

**In addition to the Final CRP Proceedings, the following Research Results were published by participants during the CRP:**

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**OTHER OUTPUTS:**

Enhanced networking and collaboration among and between natural enemy researchers, and laboratories and institutions working on enhancing biological control of pest insects.

Capacity building in the biological control of insect pests.

Fellowship support for some researchers to obtain specific training in other Member States.

Focused international attention on the recruitment of new researchers into the use of nuclear techniques in biological control.

**Various degrees and postdoctoral positions resulted from students/research groups participating in this CRP:**

| Country | B. Sc. / Agr. Eng. | M. Sc. Degrees | M. Phil. Degrees | Ph. D. Degrees | Post-doc Positions |
|---|---|---|---|---|---|
| Argentina | 1 | | | | |
| Austria | | 1 | | 1 | |
| Bangladesh | | 1 | | 1 | |
| Bulgaria | | | | 1 | |
| India | | | 4 | 3 | 1 |
| Mexico | 2 | | | | |
| Pakistan | | 2 | | 2 | |
| Poland | | 1 | | 1 | |
| Turkey | | 1 | | 2 | |
| USA | | 2 | | | 1 |
| TOTAL | 3 | 8 | 4 | 11 | 2 |

**Some of the Thesis Published or under preparation:**

Andiarena, C. 2004. Uso de radiación gamma para extender el periodo de parasitación por *Spalangia endius* de pupas de *Musca domestica*. Thesis to obtain the degree in Agricultural Engineering. Facultad de Agronomia, Universidad de Buenos Aires. p.38.

Celmer, K. Effects of Gamma Radiation to Some Biological Properties of Mediterranean Flour Moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and Egg Parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogramma-tidae). PhD. Thesis, University of Warsaw.

Celmer, K. Investigation of Some Biological Properties of Egg Parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) Reared on Irradiated Mediterranean Flour Moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). MSc. Thesis, University of Warsaw.

Rayhan, U. 2005. Control of Uzi fly, *Exorista sorbillans* Wied., an endoprarasitoid of silkworm *Bombyx mori*, L., integrating the nuclear and other techniques. Ph. D. Thesis. Rajshahi University, Bangladesh.

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Seth, R. K. et al. 2003. Effect of low dose gamma irradiation of entomopathogenic nematodes, *Steinernema glaseri*, on their parasitization behaviour and bioinfective competence towards lepidopteran host, *Spodoptera litura*, M. Phil. Dissertation, Delhi University.

Seth, R. K. et al. 2003. Bioefficacy of entomopathogenic nematodes, *Steinernema glaseri*, vis-à-vis irradiated and MH-agonist treated host, *Spodoptera litura*, M. Phil. Dissertation, Delhi University.

Seth, R. K. et al. 2003. Effect of irradiation of lepidopteran hosts on parasitization efficacy of egg parasitoid, *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), M. Phil. Dissertation, Delhi University.

Seth, R. K. et al. Parasitoid-Host Interaction between Entomopathogenic Nematodes, *Steinernema glaseri* and host, *Spodoptera litura* vis-à-vis gamma irradiation other Control Measures. Ph. D. Thesis, Delhi University.

Seth, R. K. et al. Effect of low dose radiation and their potential use in biological and parabiological modes of lepidopteran pest suppression. Ph. D. Thesis, Delhi University.

Seth, R. K. et al. Use of nuclear techniques in influencing efficacy of parasitoid *Trichogramma chilonis* Ishii for controlling lepidopteran pests**.** Ph. D. Thesis, Delhi University.

Tillinger, N. A. 2002. The effect of different doses of Cobalt radiation on the function of associated polydnavirus in the host-parasitoid system *Lymantria dispar* (Lep., Lymantriidae) and *Glypanteles liparidis* (Hym., Braconidae). Thesis, University Vienna, Austria, 152 pp.

**8. Effectiveness of the CRP**

a) In reaching the Specific Objective "To assess the potential roles nuclear techniques can play in improving production, and facilitating trade and use of quality biological control agents in integrated pest management." The CRP successfully met its specific objectives. Many potential roles were identified and investigated including (1) improving rearing media/host and utilising by-products of mass rearing facilities, (2) addressing some shipping-related problems concerning live hosts for natural enemies, (3) using of sub-sterile or sterile hosts or prey in the field as supplemental food to natural enemies, (4) facilitating the complementary and integrative use of biological control with the SIT or inherited sterility, and (5) exploring the potential for using reproductively inactivated exotic agents for confirmation of host specificity of potential biological control agents, and for exploration and collection of new natural enemies.

b) <u>In contributing towards Overall (i.e. Agency Project) Objective</u> "To increase the cost-effectiveness, trade and safety in the use of biological control agents of agricultural insect pests". The overall objective was met. Nuclear techniques investigated during the CRP have been established in practical application to reduce the cost of pest biological control, reduce the dependency of insecticides, and increase the effectiveness and safety in the use of natural enemies. Producers of natural enemies, industry, growers, scientists, and regulatory agencies are adopting many of these techniques.

c) <u>Factors, if any, which adversely affected the effectiveness of the CRP</u>

i. *Appropriateness of the CRP*: The CRP covered the very broad field of biological control. Nevertheless, this was appropriate in view that it was the first CRP in this field and the use of nuclear technique in relation to natural enemies is a largely unexplored topic with only a limited amount of basic references available. One disadvantage of the breath of the CRP and the many different insect pests being researched was that there were fewer interactions among CRP participants than would be desirable. A more focused follow-up CRP could address this issue.

ii. *Formulation of the CRP*: The CRP was largely based on the recommendations of a consultants meeting. It was well formulated and the challenge to properly address a very broad new subject area was met by developing sub-groups, which focused respectively on the main areas of potential application of nuclear techniques in biological control.

iii. *Management problems during implementation of the CRP*:

**Location:** Two RCM had to be redirected and held in Vienna in view of unforeseen circumstances (problems to secure host country agreement in one case and war in neighbouring country in another). This represented lost opportunities to network and see activities in other countries.

**Timing**: There was some delay in holding some of the RCM because of the problems explained under location. However, this delay did not affect the effectiveness of the CRP; actually it allowed researchers to get more of the agreed work done.

iv. *Intellectual, financial and other inputs from participants*:

There were considerable inputs from most of the various participating institutions. Only the Indonesia research contract holder dropped out after two RCMs. The participants from China and Slovakia were not able to attend the final RCM, but both submitted their reports and final manuscripts.

## 9. Impacts of the CRP

1. Demonstrated the utility of sub-standard and excess material produced in mass-rearing facilities for rearing biological control organisms, thus increasing the cost efficiency of rearing operations and improving the control options and potential of area-wide management programmes.

2. Demonstrated the potential of irradiation to extend the suitability of host material for exposure to parasitoids and predators. In addition to improving the production capabilities of a rearing facility, this application opens business opportunities to ship sterile host/prey material for use and redistribution by smaller facilities producing natural enemies.

3. Increased efficiency of biological control programmes by eliminating the need to separate non-parasitized host material from parasitoid emergence containers. This additionally has opened the opportunity to apply new technologies in the management of rearing and release operations (e.g. use of chilling in collection and packaging procedures).

4. Provided an additional host specificity-testing tool (use of $F_1$ sterility) to assess the safety of lepidopteran species being considered for release in classical biological control.

5. Demonstrated the utility of irradiation to suppress, and thus elucidate, host immune responses, and to take advantage of immuno-suppressed hosts to facilitate and improve the production of natural enemies and allow the use of easier to rear factitious hosts.

6. Demonstrated the benefits of supplementation of irradiated hosts early in the season to build up natural enemy populations, or during critical periods to facilitate the survival of biological control agent populations.

7. Developed a basic tool for studying complex host-natural enemy interactions.

8. As a result of the CRP, this area of applied entomology has been added to a university curriculum.

9. Policy-makers have been made aware of the potential and benefits of this technology.

## 10. Relevance of the CRP

*New emerging thrust area*

Nuclear techniques have been found to be very relevant to this until now unidentified area. It has been recognized as an emerging thrust area. Certain related, new emerging trends have come up, such as host responses to irradiation, insect immunity, and insect allergy.

*Application in biological control (production, trade, and conservation)*

The results offer great practical application in production, trade, and conservation of natural enemies. Using radiosterilized hosts offers a means for eco-safe (risk free mode) of transport and release of natural enemies. Radiosterilized host/prey (pest) insects are environmentally safer and more effective for both inundative and inoculative releases of natural enemies. In addition, low doses of radiation can be used in enhancing production and the efficiency of natural enemies.

*Tool in basic science*

Irradiation has been shown to be a useful tool in developing an in-depth basic understanding of pest – natural enemy interactions.

## 11. Recommended Future Action by the Agency

Follow-up Activities

In conjunction with the CRP certain related and pertinent emerging research topics have been identified that merit further investigation. It is recommended that some of these new areas related to biological control and nuclear techniques be considered for developing a future CRP.

There is the need to explore various possibilities of using alternative and more cost-effective sources of irradiation to facilitate in the future the application by end-users of radiation in biological control.

*Training and Capacity Building*

The Agency should continue critically important training programmes for related insect radiation biology studies.

Efforts should continue to pursue opportunities of holding RCMs in conjunction with relevant training workshops and/or international meetings.

Furthermore, support for scientific exchanges between research institutions and action programmes will facilitate technology transfer in this field. One suggested location that could be considered for holding a related workshop is the Montpellier Institute in France.

## 12. Publication of the Results of the CRP

The final proceedings of the CRP are being peer-reviewed and edited for publication as full papers in a dedicated issue of the UK journal BIOCONTROL SCIENCE AND TECHNOLOGY.