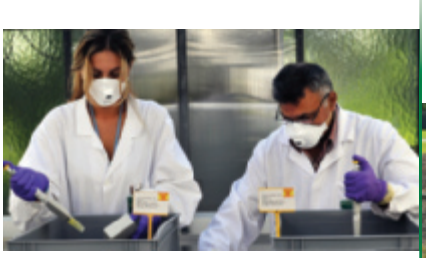
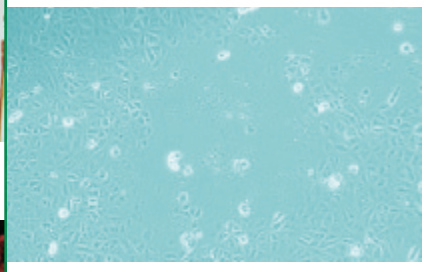
Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

FAO/IAEA
Agriculture & Biotechnology
Laboratories

Activities Report **2010**




IAEA Laboratories Seibersdorf
International Atomic Energy Agency
Vienna, Austria

FAO/IAEA Agriculture & Biotechnology Laboratories
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
International Atomic Energy Agency
PO Box 100, 1400 Vienna, Austria

Printed by the IAEA in Austria, February 2012

CONTENTS

ANIMAL PRODUCTION AND HEALTH LABORATORY

EXECUTIVE SUMMARY

Almost two thirds of the world's farm animal population is raised in developing countries where livestock production constitutes an important resource for the subsistence of more than 70% of the impoverished people living there. Animals represent an essential source of protein and contribute to the economic development of these countries and to overall food security. However, production losses caused by animal diseases, estimated to be around 20% worldwide, have a huge negative impact on livestock productivity. The Animal Production and Health Laboratory (APHL), within the Animal Production and Health Section, conducts applied research activities to develop diagnostic tools and assists in the transfer of these tools to FAO and IAEA Member States in their efforts to improve livestock productivity, ensure food security and fight against hunger.

In Africa, trypanosomiasis affects both livestock and humans, and constitutes a severe constraint to agricultural development, greatly affecting food production and economic growth. It is estimated that in Africa trypanosomiasis causes annual losses of at least US $1.3 billion. Trypanosomiasis is caused by several different species of a haemoparasite belonging to the genus *Trypanosoma*; in Africa, it is mostly transmitted by tsetse flies. At present, the control of trypanosomiasis is carried out through vector control, the use of trypanocidal drugs and the rearing of trypano-tolerant cattle. None of these is fully satisfactory. One control strategy with great potential impact, if it could be developed, is vaccination. Unfortunately, despite the many efforts that have been employed for several decades to develop a vaccine to control trypanosomiasis, no effective product is yet available. Based on the results achieved so far, APHL has begun developing a trypanosomiasis vaccine using an approach that involves irradiating the parasites with a gamma source to prevent their growth while preserving the natural structure and metabolic activity to enable efficient triggering of the host's immune system.

In 2010, APHL continued its research and development (R&D) activities on two other diseases that are rampant in Africa, the Middle East and Asia: peste des petits ruminants (PPR) and capripox. In these regions, PPR and capripox are the main killers of sheep and goats, two animal species that constitute important sources of protein and income for smallholder farmers. For PPR, APHL carried out research to validate the new cell culture system developed in 2009 to facilitate the isolation of the PPR virus (PPRV). In addition, a proficiency test (ring test) was organized to evaluate the performance of ten participating laboratories in the use of the nucleic acid amplification technique for the detection of PPRV and to provide advice. For capripox, upon request by two Member States, APHL conducted genome sequencing and molecular epidemiology analysis of their capripoxvirus (CaPV) strains to assist in the characterization of the viruses involved in the capripox outbreaks occurring in small ruminant populations despite vaccination.

In animal genetics, APHL collaborated with other institutions in the successful development of the whole-genome radiation hybrid panel (Goat RH5000), which constitutes an important tool

for the study of goat genomics and for the identification of important traits that could be used in the genetic improvement of goats. A second area of research has been the characterization of indigenous chicken breeds in the search for unique properties in immunity related genes. This has allowed the identification of markers potentially linked to the resistance of chicken to Marek's disease and avian influenza, two major viral diseases.

Finally, as part of the activities in capacity building and transfer of technologies to Member States, APHL organized two training courses: (a) Regional Training Course on Animal Health in Molecular Diagnosis, Genotyping and Phylogenetic Analyses of Avian Influenza (Bird Flu) and other Mammalian Influenza A Subtypes (20 September to 1 October 2010); and (b) Regional Training Course on Genomic DNA Preparation, Microsatellite Analyses and Sequencing (22 November to 3 December 2010).

STAFF

Name	Title	Email	Extension
Diallo, Adama	Virologist/Molecular Biologist, APHL Head	Adama.Diallo@iaea.org	28355
Malek, Massoud	Molecular Geneticist	M.Malek@iaea.org	28358
Lamien, Charles Euloge	Molecular Biologist	C.Lamien@iaea.org	28314
Winger, Eva Maria	Senior Laboratory Technician	E.Winger@iaea.org	28302
Lelenta, Mamadou	Laboratory Technician	M.Lelenta@iaea.org	28321
Pichler, Rudolf	Laboratory Technician	Rudolf.Pichler@iaea.org	28378
Francisco, Berguido	Consultant	F.Berguido@iaea.org	28356
Adombi, Caroline	Consultant	C.M.Adombi@iaea.org	28380
Slawinska, Anna	Consultant		
Lorenz, Anne	Secretary	A.Lorenz@iaea.org	28274
Swoboda, Elisabeth	Secretary	E.Swoboda@iaea.org	28362

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Trypanosomiasis: Development of an irradiated trypanosome vaccine

In 2010, APHL started a project that aims at developing "an irradiated metabolically active but non-replicating trypanosome vaccine". This project is part of the CRP entitled The Use of Irradiated Vaccines in the Control of Infectious Transboundary Diseases of Livestock.

To determine its feasibility, this approach will be tested first on *Trypanosoma evansi*. This parasite, transmitted by biting insects of various species, is the most widely geographically

distributed pathogenic trypanosome. It causes disease in livestock in Africa, South and Central America, and Asia. Most domestic animals such as buffalo and cattle infected with *T. evansi* suffer from chronic diseases, and they can survive several months to several years after infection without treatment. Moreover, there is experimental evidence to show that some animals can undergo self-cure from *T. evansi* infection, an event that is accompanied by qualitative and quantitative changes to their lymphocyte populations.

For this study, a recombinant *T. evansi* strain expressing the luciferase gene that was obtained from the Prins Leopold Instituut voor Tropische Geneeskunde (Antwerp), Belgium, will be used. The expression of the luciferase gene will be monitored to measure the effect of irradiation on the development of the parasite. Initial studies have focused on the stable propagation of this modified trypanosome along with the parent strain from which it is derived: estimating the optimal cell density, culture conditions, freezing of backups of the parasites in liquid nitrogen, viability checking of backups by resuscitation and re-cultivating in vitro. Upon modification of the cell culture medium and optimization of the parasite density, a pattern of stable growth of the trypanosomes has been achieved. Microscopical observations during the process of cultivation have shown that the recombinant strain is not as robust as the wild type. Plaques of dead parasites are found earlier, and in higher numbers, than with the wild type. This should be taken into consideration after viability studies upon irradiation, since the recombinant strain does not reflect the field situation.

Peste des petits ruminants: Development of diagnostic and marker vaccine tools

New cell line for the isolation of PPRV

Isolation of pathogens is a key step in the diagnosis of infectious diseases. In the case of PPR, the virus which is responsible for this disease is isolated in vitro on epithelial cells. These cells are not the natural targets for this virus, which instead enters lymphoid tissue cells, where it grows readily. However, this cell type is difficult to maintain in culture. The lymphotropic affinity of this virus is due to the presence of a protein on the surface of the lymphoid cells that the virus uses as a receptor to facilitate its entry into the host. In 2009, we succeeded in integrating the gene of this protein of goat origin into the genome of a monkey epithelial cell and demonstrated in a preliminary study that the resultant cell is suitable for the isolation of PPRV in vitro. In 2010, we validated these new cells for the isolation of the PPRV from pathological samples from sick sheep and goats in Nigeria and Côte d'Ivoire. With those samples we showed that PPRV can be isolated in the new cell line from day 1 to day 10 after infection without any subsequent blind passage. The cytopathogenic effect (CPE) was characterized by the appearance of large syncytia in the cell layer (see Fig. 1).

However, with the same samples, a CPE related to a virus infection could be detected only after 11 blind passages, i.e. 11 weeks in monkey epithelial cells that are usually used for PPRV isolation in vitro but that do not express the virus receptor. This finding will certainly constitute an important element in the future for the diagnosis of PPR by facilitating the isolation of PPRV from pathological specimens. The validation of this new cell line will continue in 2011 with more samples that have now been received from several other African and Asian countries. In the meantime, these cells have already been sent for testing to some partners in Member States.

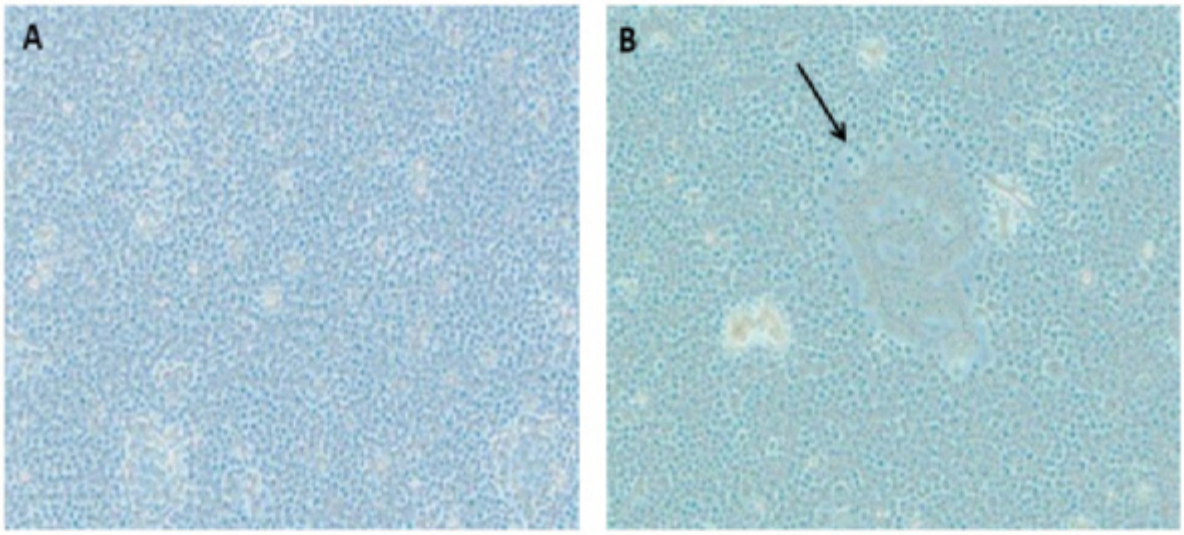


FIG. 1. E6-Slam cells: A – non-infected control cells; B – cells infected with pathological PPRV lung specimen. Arrow shows syncytium.

Validation of PPRV nucleic acid amplification technique

In 2008, the IAEA launched a coordinated research project (CRP) entitled The Early and Sensitive Diagnosis and Control of Peste des Petits Ruminants (PPR) (CRP D32026) with the overall objective of developing, validating and transferring to Member States sensitive, specific and rapid tests for the diagnosis of PPR to help them better manage and control this transboundary animal disease. One of the specific objectives of this project is to evaluate and validate the gene amplification, reverse transcriptase-polymerase chain reaction (RT-PCR) methods currently in use for the diagnosis of PPR. In that frame, in 2010 APHL organized an inter-laboratory proficiency ring test on the RT-PCR technique for PPR diagnosis. All ten project contract holders participated in this ring test. Based on the results obtained, there is a clear need to help some counterparts in improving the implementation of the test. Following recommendations to enable them to troubleshoot their various problems, another ring test will be organized in 2011.

Study of the PPRV protein–protein interaction: Mapping the nucleoprotein (N) binding site on the phosphoprotein (P)

In 2003, the APHL embarked on an EC and Wellcome Trust Foundation supported collaborative project to develop a PPR marker vaccine. Within that project, APHL was tasked with studying virus protein–protein interactions to identify the nucleoprotein (N) zone(s) that are not essential for virus growth and that therefore can be used as insertion site(s) for a potential vaccine marker. In previous years, we reported the results of studies of the nucleoprotein N self-interactions, its interaction with the virus matrix protein, the protein which drives the maturation of the virus, and its interactions with the phosphoprotein (P), the viral protein which is a cofactor of the enzyme responsible for the replication of the viral genome. In particular, in 2008, the binding sites of P on N were identified. In 2009, the N and P interaction studies continued with the identification of the binding sites of N on P.

During 2010, using a newly developed monoclonal antibody anti-P-PPRV, 2B11, the results of the identification of the binding sites of the phosphoprotein (P) on the nucleoprotein (N) were further confirmed by means of peptide ELISAs: a plate was coated with different peptides covering the full N. The binding of P to the peptides was detected by the monoclonal antibody anti-P. In using this approach, we identified four peptides to which P is binding. Those peptides, potential binding sites of P, are located in the central and end parts of N (see Fig. 2).

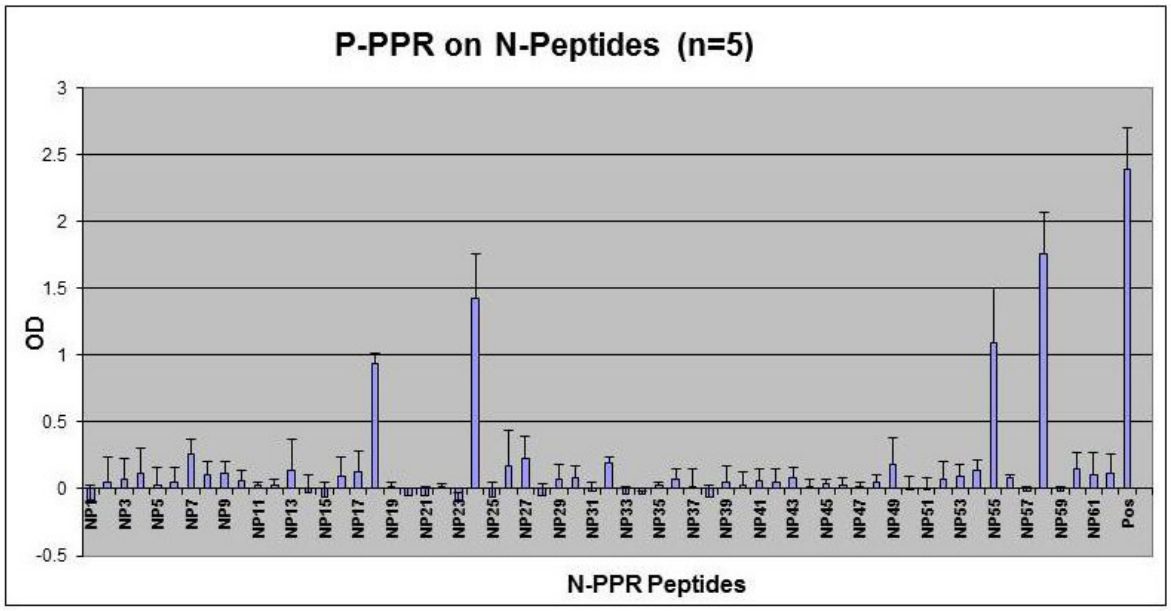


FIG. 2. Histogram representing the binding of PPRV P protein to 62 PPRV N overlapping peptides as quantified by ELISA.

Capripox: Molecular epidemiology and development of diagnostic tests

Development of an ELISA test for capripox disease

Currently, the serological diagnosis of capripox diseases is carried out by two methods — immunofluorescence and serum neutralization tests — that are not compatible with the analysis of high numbers of test samples. To address this need for an assay that can be used for high throughputs of test samples, APHL has been working since 2007 to identify genes that encode suitable immunogenic proteins for the development of a capripox ELISA based on recombinant proteins. In 2010, five CaPV genes were identified, cloned and used to express recombinant proteins. The preliminary screening of these proteins was performed using serum from small ruminants (sheep and goats) that were experimentally or naturally infected with CaPV. Screening by Western blot revealed that two of these proteins could recognize serum from CaPV infected animals. Additional evaluation of these proteins using ELISA indicated that they represent good candidate recombinant antigens for a capripox ELISA. Further studies are on the way to better define their performance.

Differentiation of capripoxviruses by real-time PCR

The use of rapid and early detection tools is crucial for enabling timely decisions to be taken to stop a given disease from spreading. Such tools have a higher value when, in addition, they can help identify the strain or subgroup of the circulating pathogens, since this information

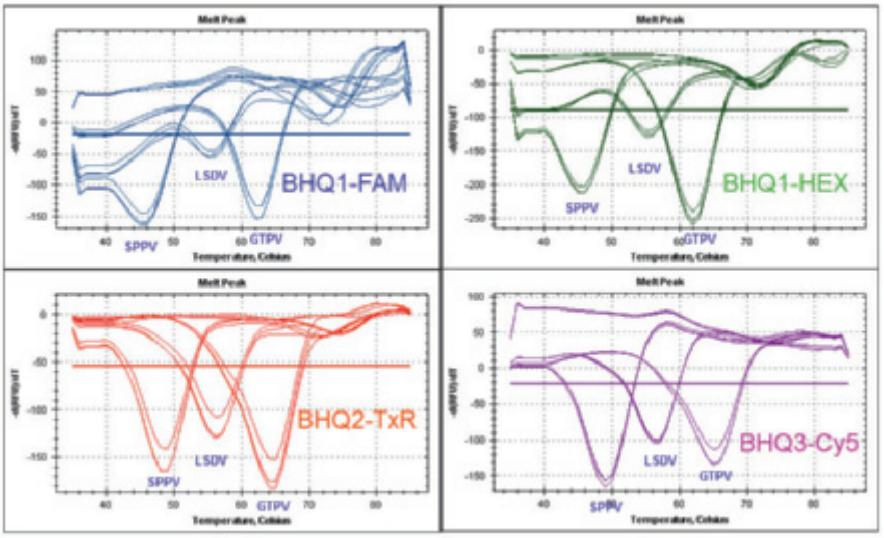


FIG. 3. Use of the Bio-Rad CFX real-time PCR system for CaPV genotyping using the new APHL real-time PCR method (quencher induced fluorescence shutdown PCR).

can indicate the strain that needs to be used for vaccination in a given area. At APHL, a real-time method to produce such a tool, known as FRET assay, has been developed and the results have been published. Despite its good performance, the major drawback of this method is that it needs specialized types of real-time PCR machines. To overcome this limitation, APHL has been working since 2009 on an alternative to the FRET technique. This new method can be performed on all real-time PCR machines currently available on the market (Fig. 3). In 2010, this method was further improved for the differentiation of CaPV strains. A first version of a programme designed to handle data produced by this new method was evaluated for further improvement.

The assay specificity was determined, and the results showed that it was highly specific to CaPV; moreover, no unwanted nucleic acids were detected. The limit of detection of the assay was determined for each of the three CaPV genus members: sheep poxvirus, goat poxvirus and lumpy skin disease virus. The new assay was compared with the previous FRET assay, and the results show that it performs even better than the original technique in detecting CaPV from clinical specimens.

Molecular epidemiology

APHL has undertaken a study of the full genome of several field and vaccine strains of CaPV in order to identify genes that are involved in determining their host specificity and virulence. From 2007 to 2009, 11 full genomes were sequenced. In 2010, the genomes of two batches of the Algerian vaccine strain and one field isolate were fully sequenced. This was done partly at the request of our counterpart in Algeria to assist in understanding the underlying reasons for the emergence of capripox in vaccinated flocks. The analysis of these genomes showed a clear difference between the Algerian vaccine strain and the field strain that was tested. Also, surprisingly, differences were observed between the two different batches of the vaccine strain. Further work is planned with our Algerian colleagues to determine if the differences observed between two batches of the same vaccine strain could affect the efficiency/residual pathogenicity of the vaccine.

Other studies concerned CaPV isolates, with complex epidemiological backgrounds, that were received from Ethiopia. The counterpart in this Member State had asked for the identification

of the genotype of virus strains that had been sent to APHL. Based on the nucleic acid based techniques, developed at the APHL, it was possible to show evidence of cross-infections in the CaPV isolates:

— Two samples from sheep in two different locations were found to be goat poxviruses.
— Three samples from cattle in two different collection areas were found to be sheep poxviruses.

Animal genetics

Goat genome mapping

APHL collaborated with other institutions in a project for the development and characterization of a goat (*Capra hircus*) whole-genome radiation hybrid (RH) panel (goat RH5000). One of the objectives of this project was to provide a unique tool for the study of goat genomics to identify important traits for use in the genetic improvement of goats. Additionally, this tool will be useful to the entire research community by making it possible to carry out comparative genomic analyses with other species. The development of an RH map for the goat will allow researchers who discover a phenotype of interest in the goat to use it as a model for comparative analysis and gene discovery. Because the goat has adapted to virtually every type of environment and has many biomedical conditions similar to those of humans and other ruminants, it will be a valuable resource for this comparative genomic approach. For the construction of this RH panel, cells from a goat donor were irradiated with a ^{60}Co source for a total dose of 5000 rad. These cells were then fused with recipient Chinese hamster TK−cells (A23) to generate the creation of some 130 RH colonies. Some of these hybrids will be used for further goat genome mapping studies.

Genetic characterization of indigenous chicken breeds in the search for the unique properties of the immune related genes

Domestic chicken is one of the most important animal species worldwide. Currently, the world poultry market is facing recurrent outbreaks of contagious diseases, which provide a serious health and economic threat. Commercial chicken breeds that serve as the most common genetic resource currently available are likely to lose important traits for resistance through a long term process of one sided selection for production traits. In 2010, APHL started a programme on the genetic characterization of indigenous chicken breeds to identify markers that could be involved in resistance to some infectious diseases.

First, the major histocompatibility complex (MHC) was investigated, since resistance to certain contagious diseases (e.g. Marek's disease) is strongly associated with the MHC variant. Secondly, single nucleotide polymorphisms (SNPs) within some key genes of the immune response were also studied, including the myxovirus resistance gene (Mx) that is reported to confer resistance to avian influenza virus. Another gene investigated was the interleukin 2 gene (Il-2), which is an immunoregulatory cytokine driving the immune system towards macrophage activation and antibody production.

CAPACITY BUILDING

Fellowships

Mr Davaasuren Batdorj from Mongolia was a TC supported fellow in APHL for 6 months (18 January to 17 July 2010) for virus genotyping by real-time PCR and gene sequencing and recombinant protein expression.

Ms Amel Bahlel–Omani from the Central Laboratory of the Institute of Veterinary Medicine in Algiers, Algeria, was hosted for 2 months (18 January to 17 March 2010) to study the molecular epidemiology of Algerian isolates of CaPV.

Ms Mechtilda Byamungu from the Tsetse and Trypanosomiasis Research Institute, Tanga, United Republic of Tanzania (June 2009 to February 2010) was trained on the isothermal nucleic acid amplification technique for the identification of trypanosomes.

Mr Rawad Bashur, a student at the University of Paris Jussieu, was an intern in APHL from August 1 to September 2010. He was trained on molecular techniques for PPRV detection.

Mr Taolo Tebogo Apadile, Botswana, was a TC supported fellow in APHL from 1 June to 31 August 2010, for training on the laboratory information management system (LIMS)

Training courses

In 2010, APHL organized two training courses at the IAEA Laboratories at Seibersdorf:

(1) Regional Training Course on Animal Health in Molecular Diagnosis, Genotyping and Phylogenetic Analyses of Avian Influenza (Bird Flu) and other Mammalian Influenza A Subtypes (20 September to 1 October 2010).
This course aimed at enhancing knowledge of early diagnosis and epidemiology tools of highly pathogenic avian influenza and other mammalian influenza A subtypes (involving the use of nuclear and nuclear related and molecular technologies), and bioinformatics tools that are required to analyse data. The targeted outputs were:

- Increased knowledge and skills in early and rapid diagnosis, genotyping and phylogenetic of avian influenza and other mammalian influenza A subtypes;
- Strengthened ability to use molecular and nuclear related tools for the diagnosis and differentiation of avian influenza and other mammalian influenza A subtypes;
- Enhanced capacity to troubleshoot and interpret results.

(2) Regional Training Course (RTC) on Genomic DNA Preparation, Microsatellite Analyses and Sequencing (22 November to 3 December 2010).
This course aimed at enhancing knowledge about highly pathogenic avian influenza (molecular genetics tools by use of nuclear and nuclear related and molecular technologies), genomic DNA preparation, microsatellite analyses and sequencing. The course was attended by 14 participants from ten Member States from RER5015 participating countries, one participant from Burkina Faso and two from Ethiopia. The goal was to train partners in molecular genetic analysis.

PUBLICATIONS

Adombi, C.M., Lelenta, M., Lamien, C.E., Shamaki, D., Koffi, Y.M., Traoré, A., Silber R., Couacy-Hymann, E., Bodjo, S.C., Djaman J.A., Luckins, A.G. and Diallo, A. (2011). Monkey CV1 cell line expressing the sheep-goat SLAM protein: a highly sensitive cell line for the isolation of peste des petits ruminants virus from pathological specimens. Journal of Virological Methods, 173 (2): 306–313.

Ajmone-Marsan, P. and the GLOBALDIV Consortium [Malek, M.] (2010). A global view of livestock biodiversity and conservation — GLOBALDIV. Animal Genetics 41 (Suppl. 1): 1–5.

Boettcher, P.J., Tixier-Boichard, M., Toro, M.A., Simianer, H., Eding, H., Gandini, G., Joost, S., Garcia, D., Colli, L., Ajmone-Marsan, P. and the GLOBALDIV Consortium [Malek, M.] (2010). Objectives, criteria and methods for using molecular genetic data in priority setting for conservation of animal genetic resources. Animal Genetics, 41 (Suppl. 1): 64–77.

Groeneveld, L.F., Lenstra, J.A., Edling, H., Toro, M.A., Scherf, B., Pilling, D., Negrini, R., Finlay, E.K., Jianlin, H., Groeneveld, E., Weigend, S. and the GLOBALDIV Consortium [Malek, M.] (2010). Genetic diversity in farm animals — a review. Animal Genetics, 41 (Suppl. 1): 6–31.

Joost, S., Colli, L., Baret, P.V., Garcia, J.F., Boettcher, P.J., Tixier-Boichard, M., Ajmone-Marsan, P. and the GLOBALDIV Consortium [Malek, M.]. (2010). Integrating geo-referenced multiscale and multidisciplinary data for the management of biodiversity in livestock genetic resources. Animal Genetics, 41 (Suppl. 1): 47–63.

Lamien, C.E., Le Goff, C., Silber, R., Wallace D.B., Gulyaz, V., Tuppurainen, E., Madani, H., Caufour P., Adam, T., El Harrak, M., Luckins A.G., Albina, E. and Diallo A. (2010). Use of the Capripoxvirus homologue of Vaccinia virus 30 kDa RNA polymerase subunit (RPO30) gene as a novel diagnostic and genotyping target: Development of a classical PCR method to differentiate Goat poxvirus from Sheep poxvirus. Veterinary Microbiology. doi:10.1016/j.vetmic.2010.09.038.

Lamien, C.E., Lelenta, M., Goger, W., Silber, R., Tuppurainen, E., Matijevic, M., Luckins, A.G. and Diallo, A. (2011). Real-time PCR method for simultaneous detection, quantitation and differentiation of capripoxviruses. Journal of Virological Methods, 171 (1): 134–140.

EXTERNAL COOPERATION AND PARTNERSHIPS

Institute	Topic
Institute for Animal Health (IAH), Pirbright Laboratory, United Kingdom	Capripox
National Animal Health Diagnostic and Investigation Center (NAHDIC) and the National Veterinary Institute (NVI), Ethiopia	
Institut National de la Médecine Vétérinaire (IMV), Laboratoire Central Vétérinaire, Algeria	
Centre de Coopération Internationale en Recherche Agronomique et le Développement (Cirad), France	Peste des petits ruminants and capripox
Laboratoire Central Vétérinaire (LCV), Mali	
High Security Laboratory, Institute for Veterinary Disease Control, Austrian Agency for Health and Food Security (AGES), Austria	Exotic animal diseases (including capripox and peste des petits ruminants)
The Prins Leopold Instituut voor Tropische Geneeskunde, Belgium	Trypanosomiasis

FOOD AND ENVIRONMENTAL PROTECTION LABORATORY

EXECUTIVE SUMMARY

The aims of the Food and Environmental Protection Laboratory (FEPL), as a component of the Food and Environmental Protection (FEP) Section, are to provide assistance and support to developing countries in their efforts to ensure the safety and quality of food and agricultural commodities, thereby safeguarding the health of consumers and facilitating international trade. The focus of the FEPL's work is on improving Member States' laboratory and regulatory practices and methodologies. The main areas of activity in pursuit of the FEPL objectives are applied R&D, technology transfer and support for the development of international standards and guidelines.

The laboratory activities support a holistic food safety approach in Member States, with food traceability now becoming a major focus. A consultants meeting was held in 2010 to develop a CRP on the Implementation of Nuclear Techniques to Improve Food Traceability and to help define future FEPL work. To support the CRP and technical cooperation (TC) projects in Member States, research commenced in 2010 in the FEPL to investigate the use of robust, affordable, transferable techniques such as laser spectroscopy to measure $^{18}O/^{16}O$ and $^{2}H/^{1}H$ isotopes in water extracted from fruit and vegetables to provide information on their origin.

Research on the control of food contaminants included the coordination of a CRP on Integrated Analytical Approaches to Assess Indicators of the Effectiveness of Pesticide Management Practices at a Catchment Scale, for which the third research coordination meeting (RCM) was held in 2010. Direct laboratory support was provided through the development and transfer to CRP participants of bioassays and methods for characterizing pesticide behaviour in soil using ^{14}C labels. An investigation using ^{14}C labelling was also carried out to examine the potential for contamination of food and feed crops by transfer of naturally produced plant toxins from the environment. The results indicated that translocation of plant toxins is a potential health risk to consumers.

In an inter-agency project focusing on sub-Saharan Africa, a method was developed for the simultaneous determination of a range of trypanocidal veterinary drugs, to be used for quality control purposes and to combat the use of counterfeit drugs. The use of counterfeit drugs is a cause of significant food safety and food security problems due to the ineffective treatment or poisoning of livestock, the development of drug resistance in disease vectors and the presence of unwanted chemicals in animal derived food products. The new method allows the transfer of a single protocol that can be used for quality control of a number of different drug formulations, primarily in African Member States.

The FEPL was involved in an advisory capacity in two EU projects focusing on the development of new multiple-contaminant detection methods in food. Several simple, inexpensive, robust methods have been developed, which are suitable for transfer to Member States under the IAEA TC mechanism.

Presentations on the FEPL research and achievements were given at five international conferences and seminars, and the FEPL was involved in the scientific committees for three future major international conferences.

Training activities by the FEPL in 2010 to support technology transfer under various TC projects and CRPs included four courses or train-the-trainers workshops in Member States and a practical course in the FEPL. A total of approximately 100 developing country participants were trained. In addition, two interns were trained at the FEPL, and five new eLearning courses were developed and added to the FEP eLearning site.

Publications by the FEPL included seven papers in peer reviewed journals and conference proceedings, the preparation of four book chapters and the publication of a book on sampling for mycotoxin control.

STAFF

Name	Title	Email	Extension
Cannavan, Andrew	Laboratory Head	A.Cannavan@iaea.org	28395
Maestroni, Britt Marianna	Food Scientist	B.Maestroni@iaea.org	28398
Islam, Marivil	Analytical Chemist	M.Islam@iaea.org	28394
Rathor, Mohammad Nasir	Laboratory Technician	M.Rathor@iaea.org	28397
Kist, Alla	Laboratory Technician	A.Kist@iaea.org	28393
Sasanya, James Jacob	Consultant	J.Sasanya@iaea.org	28327
Jandric, Zora	Consultant	Z.Jandric@iaea.org	28373
Wimberger, Tamara	Team Assistant	T.Wimberger@iaea.org	28267

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Technology development for food traceability

Initial work on food traceability using cavity-ring-down spectroscopy (CRDS) commenced during 2010 in the FEPL. The objective of this work is to develop methodologies for the traceability and authentication of food that can be transferred and applied in many developing countries. The CRDS instrument installed in the FEPL is a stable isotope analyser based on laser absorption, which measures $^{18}O/^{16}O$ and $^{2}H/^{1}H$ isotopes in liquid water. For certain applications, this type of instrument may offer a robust and affordable

alternative to more complex techniques such as isotope ratio mass spectrometry (IRMS), especially for field work. The work carried out at the FEPL focuses on the optimization of a sample preparation methodology to obtain 'pure water' — that is, free from interfering substances — from fresh fruits and vegetables to allow accurate measurement of the isotope ratios by CRDS. Two main approaches are applied; cryodistillation of extracts obtained from homogenized fruits and vegetables, and measurement of the isotopic composition of equilibrated air from homogenized fruits and vegetables stored in airtight plastic bags.

In this context, a study of the contribution of different types of irrigation water to the $^{16}O/^{18}O$ and $^{2}H/^{1}H$ isotopic composition of paprika plants at harvest, as an indicator of the geographical origin, was started in the summer of 2010.

Paprika plants were purchased locally and transplanted into previously characterized soil. Two different types of water were used for irrigation; the $^{18}O/^{16}O$ and $^{2}H/^{1}H$ isotope ratios of water samples from the two sources were measured by CRDS and found to be clearly distinguishable. The plants were divided into two groups according to the type of water used for irrigation, and were grown in a glasshouse under controlled temperature and humidity conditions. After a two month period almost all plants started to produce fruit. At harvest, individual paprika fruits were sealed in polyethylene bags and stored in a freezer until analysis. Both water samples extracted from the homogenized paprika plants by cryodistillation and equilibrated air samples were analysed by CRDS. The analysis of the isotopic composition of the paprika is ongoing.

Control of residues and contaminants in food

During 2010, the FEPL was engaged in various projects focusing on the development of methods and procedures to assist Member States to control the occurrence of harmful residues of pesticides and veterinary drugs and contaminants such as natural plant toxins and mycotoxins in food.

Pesticide residues in food and the environment

The effective control of pesticides in food and the environment requires a holistic approach that considers all points along the food chain. The soil sorption coefficient, Kd, and the soil organic carbon sorption coefficient, Koc, are basic parameters used to describe the environmental fate and behaviour of pesticides. These coefficients indicate the strength of sorption of pesticides to soils at the water–solid interface, which influences environmental mobility and persistence. Factors that influence sorption of pesticides in soil are related to the pesticide properties — such as electronic structure, adsorption by ion exchange, water solubility and nature of application formulation — and to the soil composition (% clay, silt, sand), quantity of organic matter and pH value. The determination of sorption of pesticides in soils is important for calibrating tools such as the pesticide impact rating index (PIRI) and pesticide root zone model (PRZM) in order to generate more reliable results for assessing pesticide management practices and environmental changes such as estimates of pesticide leaching and runoff. These are important aspects of various projects carried out in Member States with assistance from the FEPL, for example, regional TC project RLA/5/053.

In order to demonstrate the application of this methodology and to facilitate technology transfer, experiments were set up at the FEPL using ^{14}C labelled carbofuran to determine the sorption of carbofuran to two different Austrian soils (Seibersdorf and the Waldviertel). The parameters Kd and Koc were calculated, and the protocol for performing the experiments was transferred to project counterparts.

The third RCM of the FAO/IAEA CRP D5.20.35, Integrated Analytical Approaches to Assess Indicators of the Effectiveness of Pesticide Management Practices at the Catchment Scale, was held at the Vienna International Centre from 6 to 10 December 2010. The meeting was attended by research contract and agreement holders from Argentina, Australia, Brazil, Bulgaria, Chile, China, Costa Rica, Ecuador, Hungary, India, Kenya, the Philippines and Sweden, as well as observers from Bolivia, Brazil, Colombia and the IAEA.

The programme of the meeting included progress reports and presentations, including a summary of the 'black box' monitoring approach in which inputs and outputs are considered without investigating the pesticide interactions at the soil–water–plant level, a review of the current regional initiatives in the context of integrated analytical approaches to assess the implementation of good agricultural practices (GAP), and a discussion of insights into minimizing agriculture non-point source contamination via rapid assessment of locally relevant data. Presentations were given on a bioassay, which was also demonstrated in the FEPL; on the application of radioisotopic techniques to estimate soil erosion at the watershed scale; on modelling of transport processes affecting pesticide movement across the landscape; and on the application of Fourier transform infrared spectroscopy. Training was provided on the use of GPS and the application of geographical information systems as well as new space–time modelling tools. FAO/IAEA web resources, including the new eLearning courses on bioindicators and applications of iPads in the laboratory, were demonstrated. RCM participants agreed on the key issues for inclusion in a draft generic guideline on integrated analytical approaches to assess indicators of pesticide management practices at a catchment scale. The full text of this guideline is currently under preparation.

Natural plant toxins in food and feed

Tropane alkaloids are toxins that are harmful to humans and animals if consumed in food or feed; atropine and scopolamine, for example, have estimated lethal doses in adult humans of 10 mg and 2–4 mg, respectively. These toxins are naturally produced by the families Solanaceae and Erythroxylaceae, comprising over 100 genera and 3000 plant species found worldwide. Variable amounts of alkaloids are produced, particularly in the seeds, which are potentially poisonous. Plants can produce 30 000 or more seeds, which are typically dispersed 1–4 m^2 from the dry seed capsules and can remain viable in the soil for more than a century. Bulk commercial grains (wheat, rye, soybeans, linseed, corn and solanaceous crops) may be contaminated by non-grain impurities (alkaloids) that coexist with the crop to be harvested. Another possibly is the uptake of toxicants from the soil to food crops, thus presenting a risk to the consumer from food types in which alkaloid toxins would not normally be expected to occur. A study was designed using radioisotopically labelled ^{14}C atropine to investigate the latter possibility.

To investigate the uptake of atropine from soil by wheat and its translocation within the plant, experiments were set up using soil spiked with non-labelled and labelled atropine. Summer wheat was grown on the treated soil. Plants were harvested at different times to investigate the time course of atropine uptake and its distribution into different plant tissues. Both extractable and non-extractable ^{14}C activity was measured in water, soil and plants. Young wheat absorbed approximately 0.42% of the initial radioactivity within 15 days, decreasing to 0.04% after 90 days, probably due to metabolism by the plant and dilution caused by the increase in the biomass. Carbon-14 atropine was also detected in collected water (0.5%). The absorption of atropine from soil and its translocation to the edible plant parts, and the observed bioconcentration factor (2.3 ± 0.04) suggest a potential route of contamination that may have implications for food safety.

Combating the use of counterfeit veterinary trypanocides in developing countries.

Counterfeit veterinary drugs such as those used against trypanosomosis, an economically devastating disease, are common in many developing Member States. Such substandard drugs — with quality and safety repercussions — present two major problems. First, inadvertent underdosing of animals due to the use of counterfeit drugs containing the incorrect dosage is ineffective and ultimately leads to the development of drug resistance in the disease causing agents. This results in increased morbidity and mortality rates in affected animals, thus increasing the economic burden and escalating poverty. Secondly, and paradoxically, there may be increased administration of the drugs when animals fail to respond to treatment as expected, resulting in poisoning of the animals. These effects contribute to food insecurity. The use of counterfeit drugs containing unknown compounds may also cause a food safety hazard in, for example, milk produced by the treated animals.

Against this background, a study was initiated to develop an analytical method that is simple, quick, robust and inexpensive to ensure quality assurance/quality control of common trypanocidal drugs. A high performance liquid chromatography method with photodiode array detection was chosen, since it may be readily affordable to Member States. The drugs studied include diminazene aceturate, isometamidium chloride hydrochloride and ethidium bromide,

as well as the antipyretic–analgesic–anti-inflammatory drug antipyrine. For the first time, the method has been optimized for analysis of all these drugs in a single analytical method.

The method was demonstrated to be suitable for the intended use by analysing commercially available trypanocidal drug formulations provided by a number of international pharmaceutical companies.

These studies are being carried out in parallel with an FAO led project in collaboration with the International Federation of Animal Health (IFAH), the Institute of Pharmacy and Biomedical Sciences of Strathclyde University (SU), and the United Nations Industrial Development Organization (UNIDO). Monographs describing the authentic drugs have been jointly developed by SU and the FEPL for endorsement by the World Organisation for Animal Health (OIE) and submission to the International Pharmacopeia for use as references against which counterfeit and poor quality drugs can be tested.

Methods for the control of multiple contaminants in food

The development of innovative methods, such as those targeting multiple food contaminants, is most effectively achieved by working within multi-disciplinary research networks. In 2010, the FEPL was involved in an advisory capacity in two such EU research projects: New Technologies to Screen Multiple Chemical Contaminants in Foods (BioCop) and Contaminants in Food and Feed: Inexpensive Detection for Control of Exposure (CONffIDENCE).

The final meeting of the Advisory Board (AB) and the Top Management Group of the EU 6th Framework Integrated Project entitled New Technologies to Screen Multiple Chemical Contaminants in Foods (BioCop) was held in Rome, Italy, 28–29 September 2010. The head of the FEPL participated in the meeting as a member of the AB.

The BioCop project focused on the development and implementation of new methods to monitor and control the occurrence of multiple chemical contaminants in foods through the use of advanced sample preparation techniques and emerging biotechnological and physico-chemical screening approaches, including novel applications of existing technologies. A wide range of techniques were developed or adapted, utilizing technologies such as transcriptomics, proteomics, molecular immunology, microarrays, biosensor technology and isotope dilution mass spectrometry, resulting in a number of tests that can detect many types of toxins in foods.

The results include methods that are suitable for routine regulatory monitoring as well as techniques that are more suited to upstream research applications to underpin food safety policy development.

The second annual meeting of the AB and the Project Management Board (PMB) for the EU 7th Framework Integrated Project, Contaminants in Food and Feed: Inexpensive Detection for Control of Exposure (CONffIDENCE), was held in Ghent, Belgium, on 3 June. The head of the FEPL participated in the meeting as chair of the AB, and co-chaired the PMB-AB meeting.

CONffIDENCE is a four year project with 17 partners from ten countries and a budget of €7.5 million, €5.8 million from the EC. The main objective of the project is the development

of novel, multiplex screening methods for a wide range of contaminants in high risk products such as fish and cereal based food and feed, and vegetables. The validated methods will be applied to provide data for risk assessment and for regulatory systems for food safety. The project should result in the development of several simple, inexpensive, robust and portable test methods to enhance food safety both within and outside Europe. Some of these technologies are potentially transferable through the IAEA TC mechanism.

The inclusion of an IAEA representative in an advisory capacity in the above and similar projects helps to facilitate the effective transfer of the technologies developed to a wider customer base, including IAEA and FAO developing country Member States that are unable to undertake the primary R&D themselves. This adds value to the project outcomes through the enhancement of food safety both within and outside the EU, and through increased potential to meet the requirements for trade between developing countries and the major trading blocks of the developed world.

CAPACITY BUILDING

The expertise available at the FEPL and the methods and techniques developed were used to support technology transfer to Member States through various training and train-the-trainers activities, both at Seibersdorf and in Member States. Training activities by the FEPL in 2010 to support technology transfer under various TC projects and CRPs included four courses or train-the-trainers workshops in Member States and a practical training course at the FEPL. Approximately 100 developing country participants were trained. In addition, two interns were trained at the FEPL, and five new eLearning courses were developed and added to the FEP eLearning site.

The capacity building activities included two meetings for decision makers. A decision makers forum focusing on the role of the analytical laboratory in food safety was held in Panama in collaboration with the Directorate of Sanidad Vegetal (DNSV), a service under the Ministry of Agricultural Development (MIDA). The FEPL also provided technical support for a training course on ‘sampling for decision making’, which took place at the University of Margarita, Venezuela. The training aimed at providing ground rules for obtaining data that can be used to aid and promote decision making and included the fundamental methods used in sampling and monitoring, but with an emphasis on practical applications.

Technical train-the-trainers workshops included a course on method development, statistics and measurement uncertainty, held at MIDA, Panama, under TC project PAN/5/019, and a regional workshop on bioassays and bioindicators, organized by the FEPL at the Instituto Biologico de São Paulo, Brazil, under TC project RLA/5/053, Implementing a Diagnosis System to Assess the Impact of Pesticide Contamination in Food and Environmental Compartments at a Catchment Scale in the Latin American and Caribbean Region. The FEPL also provided technical support and guidance for an ARCAL RLA/5/053 training course on linking soil and pesticide behaviour, held jointly with the Soil and Water Management and Crop Nutrition Section of the Joint FAO/IAEA Division, at IAEA from 15 November to 3 December 2010. The purpose of the training was to provide participants with an understanding of the link

between soil components and pesticide behaviour. A module on the measurement of the soil sorption and QuEChERS (quick, easy, cheap, effective, robust and safe sample extraction and clean-up) methodology was held at the FEPL. The focus was on increasing the participants' understanding of the current RLA/5/053 'black box' monitoring strategy. A train-the-trainers approach was used to disseminate information on how laboratories can foster GAP and help deliver the Millennium Development Goals.

The FEPL hosted two interns during the year; both started in September 2010, one for a one year period and the other for three months. The interns were trained in various aspects of the FEPL's work, including stable isotope measurements using a liquid water isotopic analyser and greenhouse experiments for food traceability, and the application of gas chromatography and liquid chromatography coupled to mass spectrometry for contaminants analysis.

Distance learning is another important and effective tool used by the FEPL to support accelerated capacity building in Member States. The following distance learning courses were developed by the FEPL in collaboration with consultants during 2010:

- iPad in the laboratory — http://elearning.iaea.org/ATutor/go.php/148
- GIS and modelling — http://elearning.iaea.org/ATutor/go.php/133
- Obtaining information for decision making: A practical approach to sampling — http://elearning.iaea.org/ATutor/go.php/155
- Mass spectrometry (Spanish version) — http://elearning.iaea.org/ATutor/go.php/105
- Bioindicators — http://elearning.iaea.org/ATutor/go.php/135

In addition to direct technology transfer to counterpart institutes in Member States through training courses, fellowships at the FEPL and expert missions, it is important that the results of the R&D work performed at the FEPL are disseminated to as wide an audience as possible to assist in capacity building. The results of research performed at the FEPL, or in collaboration with partner laboratories, were presented at several international conferences and seminars in 2010, including:

- The 6th International Symposium on Hormone and Veterinary Drug Residue Analysis, Ghent, Belgium, 1–4 June 2010;
- The 21st Annual Conference of the International Environmetrics Society (TIES), Margarita Island, Venezuela, 20–25 June 2010;
- The Korean Society of Environmental Agriculture (KSEA) 30th Anniversary International Symposium on Management and Strategy on Sustainable Environment Leading to Food Safety, Busan, Republic of Korea, 8–9 July 2010;
- NVRQS Seminar on Food Safety, National Veterinary Research and Quarantine Service, Seoul, Republic of Korea, 7–8 July 2010;
- The World Mycotoxin Forum 6th Conference, Noordwijkerhout, the Netherlands, 8–10 November 2010.

In 2010, the FEPL was also involved in planning the scientific programmes of future international conferences through the Laboratory Head's inclusion in the scientific committees of:

- International Conference on Food Integrity and Traceability Conference, Queen's University Belfast, United Kingdom, 21–24 March 2011;
- The Saskatoon International Workshop on Validation and Regulatory Analysis, Saskatoon, Canada, 19–22 June 2011;
- The Euroresidue VII Conference on Residues of Veterinary Drugs in Food, Egmond aan Zee, the Netherlands, 14–16 May 2012.

PUBLICATIONS

Cannavan, A., Kay, J and Le Bizec, B. (in press). Quality assurance and quality control. In: Chemical Analysis of Antibiotic Residues in Food, MacNeil, J.D., Wang, J and Kay, J. (Eds). Wiley, NJ, USA.

Wang, J., Cannavan, A., Dickson, L. and Fedeniuk, R. (in press). Measurement uncertainty. In: Chemical Analysis of Antibiotic Residues in Food, MacNeil, J.D., Wang, J and Kay, J. (Eds). Wiley, NJ, USA.

Maestroni, B.M. and Cannavan, A. (2011). Sampling strategies to control mycotoxins. In: Determining mycotoxins and mycotoxigenic fungi in food and feed, S. De Saeger (Ed). Woodhead Publishing Limited, Cambridge, UK, pp. 3–36.

Cannavan, A. (2010). Method validation for veterinary drug residues according to EC requirements. In: Élelmiszerbiztonság megítélési módszerei (Methods for food safety assessment), A. Ambrus (Ed). Edison House Holding, Budapest, pp. 999–1022.

Whitaker, T.B., Slate, A.B., Doko, M.B., Maestroni, B.M. and Cannavan, A. (Eds) (2010). Sampling Procedures to Detect Mycotoxins in Agricultural Commodities. Springer, New York.

Jandric, Z., Rathor, M.N., Švarc-Gajić, J., Maestroni, B.M., Sasanya, J.J., Djurica, R. and Cannavan, A. (2010). Development of a liquid chromatography — tandem mass spectrometric method for simultaneous determination of tropane alkaloids and glycoalkaloids in grains. Book of abstracts of the World Mycotoxin Forum 6th Conference, Noordwijkerhout, The Netherlands, 8–10 November 2010, 144.

Cannavan, A., Maestroni, B.M. and Byron, D.H. (2010). Food safety, integrity and traceability. Proceedings of the KSEA 30th Anniversary International Symposium on Management and Strategy on Sustainable Environment Leading to Food Safety, Busan, Republic of Korea, 8–9 July 2010, 31–34.

Sasanya, J.J, Abd-Alla, A.M.M., Parker, A.G. and Cannavan, A. (2010). Analysis of the antiviral drugs acyclovir and valacyclovir-hydrochloride in tsetse flies (Glossina pallidipes) using LC-MSMS. Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences, 878, 2384–2390.

Maestroni, B., Ferris, I., Kookana, R., Cannavan, A., Byron, D. and Dercon, G. (2010). Sampling surface waters to monitor the effectiveness of good agricultural practices at a catchment scale. Book of abstracts of the 21st Annual Conference of The International Environmetrics Society (TIES), Margarita Island, Venezuela, 20–25 June 2010, 39.

Berendsen, B., Stolker, L, de Jong, J. Nielen, M., Tserendorj, E., Sodnomdarjaa, R., Cannavan, A. and Elliott, C. (2010). Evidence of natural occurrence of the banned antibiotic chloramphenicol in herbs and grass. Analytical and Bioanalytical Chemistry, 397, 1955–1963.

Berendsen, B., Stolker, A.A.M., de Jong, J.A.M. Nielen, M.W.F., Tserendorj, E., Cannavan, A. and Elliott, C. (2010). Evidence of natural occurrence of the banned antibiotic chloramphenicol in herbs and grass. Book of abstracts of the 6th International Symposium on Hormone and Veterinary Drug Residue Analysis, Ghent, Belgium, 1–4 June 2010, 27.

Cannavan, A. and Maestroni, B.M. (2010). Analytical methodology for food safety and traceability in developing countries. Agro Food Industry Hi-Tech, supplement, Focus on Food Analysis, 21, 9–12.

EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
Veterinary Public Health Laboratory, Bangkok, Thailand	Method development and research into causes of chemical contaminants in food; technology transfer to Asia/Pacific
Laboratorios Microbioticos s/c/ Ltda, São Paulo, Brazil	Method development for food contaminants; technology transfer to Latin America
University of Costa Rica (UCR), Centro de Contaminacion Ambiental (CICA), Costa Rica	IAEA Collaborating Centre for eLearning and Accelerated Capacity Building for Food and Environmental Protection (EACB)
Institut für Lebensmittel-, Arzneimittel- und Umwelt-Analytik GmbH (ILAU), Germany	Collaboration on research activities linked directly to CRP D5.20.35, Integrated Analytical Approaches to Assess Indicators of the Effectiveness of Pesticide Management Practices at the Catchment Scale
Commonwealth Scientific and Industrial Research Organisation (CSIRO), Division of Land and Water; Groundwater Management and Site Remediation, Australia	
Ministry of Health, State General Laboratory, Department of Environmental Chemistry, Ecotoxicology, Pesticides and Radioactivity, Cyprus	

Institution	Topic
Austrian Agency for Health and Food Safety (AGES), Austria	Collaboration on accelerated capacity building for risk analysis and contaminants in food
Gartner & LVA Analytik GmbH, Austria	
Austrian Institute of Technology, Seibersdorf, Austria	Collaboration on nuclear techniques for research into the transfer of contaminants from the environment to food; collaboration on the use of stable isotope measurements for traceability of foods and animals
Ashtown Food Research Centre, Ireland	Partner laboratory in EU Project 'ProSafeBeef'
Institute of Agri-Food & Land Use, Queen's University Belfast, United Kingdom	Research and method development activities for food contaminants and food traceability
ASSET Centre, Queen's University Belfast, United Kingdom	Research activities in isotope ratio methods for food traceability
International Union of Pure and Applied Chemistry (IUPAC), Chemistry and the Environment Division	Collaboration on compendium of agrochemicals information
Waters Corporation, Milford, MA, USA	Information dissemination; food safety summits
Agilent Technologies, PA, USA	Training for Member State scientists in analytical techniques
RIKILT — Institute of Food Safety, the Netherlands	Research into causes of food contamination with veterinary drug residues
Chinese Academy of Agricultural Sciences (CAAS), Institute for Application of Atomic Energy, Department of Agro-Ecological Environment, China	Development of methodology for food traceability and residues analysis
Technical University Munich, Germany	Development of radioassay protocols
World Health Organization (WHO), Lyon Office for National Epidemic Preparedness and Response	Global survey of laboratory quality standards
World Organisation for Animal Health (OIE)	
International Federation for Animal Health (IFAH)	Quality control of trypanocidal drugs in sub-Saharan Africa
United Nations Industrial Development Organization (UNIDO)	
United Nations Office on Drugs and Crime (UNODC)	

INSECT PEST CONTROL LABORATORY

EXECUTIVE SUMMARY

The Insect Pest Control Laboratory (IPCL) is an integral part of the Insect Pest Control Section and contributes to its global objectives of increasing food security, reducing food losses and insecticide use, overcoming constraints to sustainable rural development, and facilitating international trade in agriculture commodities. The IPCL achieves these goals through the development and transfer of the sterile insect technique (SIT) package for key insect pests of crops, livestock and humans.

In 2010, work continued on the salivary gland hypertrophy virus (SGHV) that causes reduced fecundity in symptomatic *Glossina pallidipes* tsetse flies with consequently stagnating or declining colonies. Work on the dynamics of the virus indicated that horizontal transmission was the main route of contamination in colonies maintained on the membrane in vitro feeding system. Promising results in managing the virus were obtained by using a strategy of clean feeding (each fly cage receives fresh blood) and the addition of the antiviral drug valacyclovir to the blood meals.

On request from the Government of Senegal, the IPCL initiated two new colonies of *Glossina palpalis gambiensis* in support of their effort to create a zone free of the fly in the Niayes area, north of Dakar. The impact of chilling and irradiation on late stage male pupae was studied, which culminated in the development of a handling and transport protocol for these pupae. This protocol is currently being validated in the field through weekly shipments of male pupae from Burkina Faso to Senegal.

Two Mediterranean fruit fly strains, which were constructed at the University of Göttingen using modern transgenesis techniques, were evaluated. Both transgenic strains in general produced fewer adults than the wild type (non-transgenic) strain, but at the level of mass-rearing used, the strains appeared to be stable after 11 generations.

Various colonies belonging to the *Anastrepha fraterculus* and the *Bactrocera dorsalis* complexes were established. Field cage studies with *A. fraterculus* from Tucuman (Argentina), and Vacaria and Pelotas (Brazil) indicated the absence of mating barriers. In contrast, initial field cage studies between *B. dorsalis* and *B. carambolae* indicated a relatively high level of mating isolation supporting their current taxonomic status.

Significant progress was made with the further development of mass-rearing techniques for the olive fly *Bactrocera oleae*. More than 3 million eggs were collected over a period of three weeks using new production cages with a wax coated ovipositioning panel, indicating that olive fly mass-rearing is becoming a reality.

Work continued on the development of suitable mosquito mass-rearing equipment and methods for *Anopheles arabiensis* and *Aedes albopictus*. A new larval tray design was successfully tested in a new rack system that can hold 50 trays. Promising results were obtained with a

new industrial version of the larvae–pupae separator. Initial data showed the feasibility of treating eggs (rather than larvae) of the genetic sexing strain of *An. arabiensis* to remove the female sex, and similar sterility levels were obtained with X rays and gamma rays.

The IPCL provided 36 months of training in 2010 that included five fellows funded by the Department of Technical Cooperation and one cost-free fellow from Pakistan. In addition, the IPCL hosted four cost-free experts from Australia, Brazil and the United States of America (USA); two interns from the USA and Italy; and five consultants from Burkina Faso, France, Italy, Pakistan and Zimbabwe.

The IPCL continuously responds to requests for services (mainly biological material, diets, DNA, etc.) from Member States. In 2010, the IPCL supplied tsetse fly pupae to five institutes in Slovakia, South Africa, the United Kingdom and the USA, and pupae and eggs of the olive fly, melon fly, South American fruit fly, Mexican fruit fly and the Mediterranean fruit fly (VIENNA 8 strain) to 14 institutes in Argentina, Australia, Croatia, Czech Republic, Germany, Israel, Italy, Mauritius, the Netherlands, Singapore, Spain and the United Kingdom.

STAFF

Name	Title	Email	Extension
Vreysen, Marc	Laboratory Head	M.Vreysen@iaea.org	28404
Abd Alla, Adly	Virologist (Tsetse Flies)	A.M.M.Abd-Alla@iaea.org	28425
Franz, Gerald	Geneticist (Plant Pests)	G.Franz@iaea.org	28419
Gilles, Jeremie	Entomologist (Mosquitoes)	J.Gilles@iaea.org	28407
Jessup, Andrew	Entomologist (Plant Pests)	A.Jessup@iaea.org	28413
Parker, Andrew	Entomologist (Tsetse Flies)	A.G.Parker@iaea.org	28408
Yamada, Hanano	Junior Professional Officer (Mosquitoes)	H.Yamada@iaea.org	28421
Targovska, Assia	Senior Laboratory Technician	A.S.Stamenova@iaea.org	28445
Wornoayporn, Viwat	Senior Laboratory Technician	V.Wornoayporn@iaea.org	28450
Adun, Henry	Laboratory Technician	H.Adun@iaea.org	28414
Ahmad, Sohel	Laboratory Technician	Sohel.Ahmad@iaea.org	28451
Marin, Carmen	Laboratory Technician	C.Marin@iaea.org	28446
Mohammed, Hasim	Laboratory Technician	A.H.Mohammed@iaea.org	28411

Name	Title	Email	Extension
Schorn, Elisabeth	Laboratory Technician	E.Schorn@iaea.org	28403
Soliban, Sharon	Laboratory Technician	S.Soliban@iaea.org	28427
Dammalage, Thilakasiri	Laboratory Attendant	T.Dammalage@iaea.org	28452
Ottilie, Ibanschitz	Laboratory Attendant	O.Ibantschitz@iaea.org	28444
Edgardo, Lapiz	Laboratory Attendant	E.Lapiz@iaea.org	28412
Misiaszek, Grzegorz	Laboratory Attendant	G.Misiaszek@iaea.org	28454
Sto. Tomas, Ulysses	Laboratory Attendant	U.Sto.Tomas@iaea.org	28455
Kurtulmaz, Elsa,	Data Entry Clerk	E.Kurtulmaz@iaea.org	28423
Wimberger, Tamara	Team Assistant	T.Wimberger@iaea.org	28267

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Tsetse

Colonies

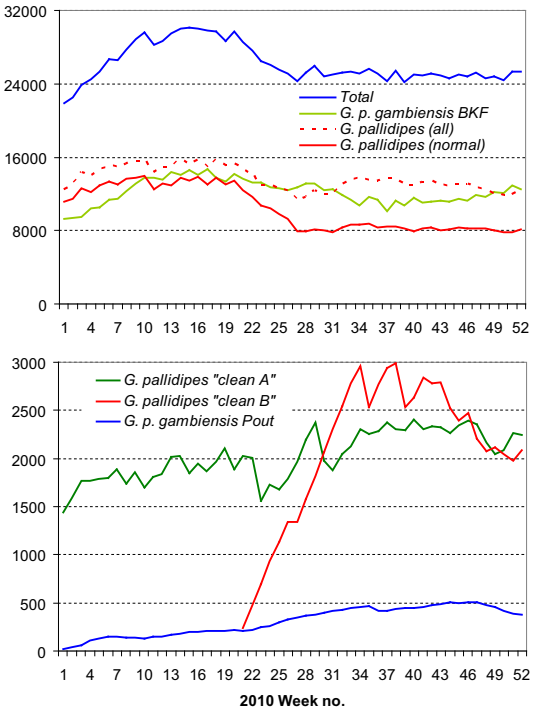


FIG. 4. Number of flies of different colonies of tsetse flies maintained at the IPCL.

The IPCL continues to maintain colonies of tsetse flies according to the needs and requests of FAO and IAEA Member States (Fig. 4). For several years, a colony of *Glossina pallidipes* (8000 producing female flies in 2010) has been maintained to provide biological material for research on SGHV. The work is done in support of tsetse projects in Ethiopia and other Member States in Eastern Africa that aim at using the SIT as part of their control efforts. In addition to the main *G. pallidipes* colony, two 'clean feeding' colonies of this species were initiated in 2010 to assess the effect of this virus management technique (see below).

In 2009, a request was received from the Government of Senegal to carry out research in support of efforts to create a zone free of *Glossina palpalis gambiensis* in the Niayes area, north of Dakar. To be able to respond to this request, a colony of the target species was initiated with 8000 pupae received from the Centre International de Recherche-Developpement Sur l'Elevage en Zone Subhumide (CIRDES) in Burkina Faso (BKF). In 2010, the BKF colony was maintained at a level of 12 000 producing females to provide all the insects required for the experimental work.

In addition to the BKF colony, a *G. p. gambiensis* colony was initiated with pupae from the target area in Senegal (SEN). Wild female flies were collected weekly from the Niayes in Senegal and transferred to an insectary in Dakar, where the female flies were maintained for pupae production. Between October 2009 and September 2010, a total of 2185 pupae were shipped from Dakar to the IPCL. In December 2010, the SEN colony had reached a size of 450 females and had become self-sustaining. Initiation of a tsetse colony from wild flies is very challenging, but due to close collaboration and intense interaction of the IPCL staff with the counterparts in Senegal the SEN colony was successfully established.

Glossina palpalis gambiensis

The Government of Senegal opted not to develop its own mass-rearing capacity, and to procure the flies from the CIRDES in Burkina Faso, where a colony of the target species has been maintained since the 1980s. To enable shipments of male pupae from BKF to SEN, adequate handling and transport protocols needed to be developed. Female flies need to be retained in the colony, and male and female *G. p. gambiensis* can only be separated at the adult stage. Adult sterile males are, however, too fragile to be shipped such a long distance. It was therefore hypothesized to exploit the difference in the pupal period of male and female pupae followed by the chilling of the male pupae to delay emergence. Work was initiated to assess the effects of chilling (10, 12.5 and 15°C), irradiation dose and the combination of both on male pupae of different age. Parameters such as pupal development, emergence rate, male survival, insemination capacity and their mating performance in field cages were investigated. This research resulted in a handling and transport protocol that is currently being validated in Burkina Faso and Senegal.

Using sterile male insects in an SIT programme that originate from a different geographical area than those of the target area requires an assessment of their mating compatibility. It is essential for the success of a programme that mating barriers between the used and targeted strain are absent. Mating studies, undertaken in field cages that mimic closely the natural environment, revealed random mating between the SEN and BKF strains of *G. p. gambiensis*.

Salivary gland hypertrophy virus

Area-wide integrated pest management (AW-IPM) programmes that incorporate an SIT component require a thriving colony of the target species to provide sufficient numbers of males for sterilization and release. Development of a sizable *G. pallidipes* colony for the tsetse project in Ethiopia has been hampered by a high prevalence (up to 40%) of salivary gland hypertrophy (SGH) caused by the SGHV (Fig. 5). Symptomatic flies have reduced fecundity causing colony stagnation or decline.

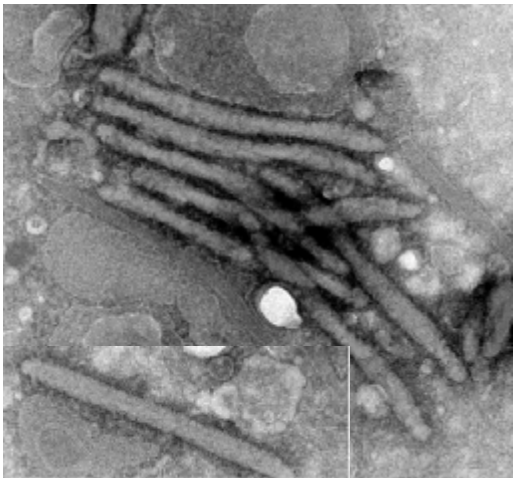


FIG. 5. Salivary gland hypertrophy virus particles.

Work on the development of suitable virus management strategies was initiated several years ago and continued in 2010. The complete genome

of the virus was sequenced, which opened avenues for PCR and quantitative PCR techniques to study the dynamics of the virus. These studies revealed that both horizontal and vertical transmission occurs, but that horizontal transmission (from fly to fly through feeding) is the main mode in colony flies, whereas in nature vertical transmission (from mother to progeny) seems more important. Colony flies become infected with the virus through the ingestion of contaminated blood that they absorb when using an in vitro membrane feeding system. A strategy of 'clean feeding' was therefore tested, whereby the blood offered to the flies was only used once for each cage of flies. This strategy resulted in a significant reduction of the prevalence of SGH.

A second strategy being investigated is the use of antiviral drugs. Research continued to assess the impact of the two drugs acyclovir and valacyclovir on the prevalence of SGH and the virus. Feeding *G. pallidipes* colony flies for two years on blood mixed with the drug valacyclovir resulted in acceptable productivity and reduced prevalence of SGH and SGHV load. Tests have been initiated to screen 15 other antiviral drugs.

Other strategies to manage the virus in the colony, such using as RNAi technology to silence the expression of certain genes or neutralizing the virus infection with specific antibodies, are being investigated, but results so far are not conclusive.

Fruit fly genetic sexing

Over the last two decades, the IPCL has been the driving force behind the development of genetic sexing strains (GSSs) for the Mediterranean fruit fly *Ceratitis capitata*. The development of these GSSs allowed the removal of the female flies from the production line and the release of only male flies. This greatly increased the efficiency of the released males in the field and improved the cost effectiveness of the rearing, handling and releasing components of the SIT. The VIENNA 8 GSS that carries a white pupae (*wp*) and temperature sensitive lethal (*tsl*) mutation is now being used in all Mediterranean fruit fly facilities around the world.

The development of these GSSs requires an inducible lethal factor and a linkage to the sex. Both classical genetics and modern transgenesis can be used to construct these strains. In recent years, the IPCL has been involved with screening and evaluating the performance of some transgenic strains (see activity report 2008). In 2010, two transgenic strains of the Mediterranean fruit fly, developed by the University of Göttingen, were evaluated. Both transgenic strains, which are being developed with the aim of inducing sterility in a population without using radiation, produced in general 10% fewer adults than the wild type (non-transgenic) strain. At the level of mass-rearing used, the strains appeared to be stable (after 11 generations), although both strains seemed to suffer from the presence of the transgene.

In addition, two strains of the Mediterranean fruit fly being mass-reared in Guatemala and Hawaii were analysed with respect to their genetic status. It appeared that both strains contained a mixture of features that were only present in the VIENNA 7 and VIENNA 8 GSSs. The construction of a new strain was initiated using the *wp* and *tsl* mutations of the VIENNA

8 GSS during outcrosses with a Guatemalan wild strain. Work was likewise continued with testing a GSS of *Bactrocera dorsalis* and with the construction of a new GSS of the melon fly *Bactrocera cucurbitae*.

Fruit fly mass-rearing and quality control

Colonies

Fruit flies are among the most severe pests of fruit commodities in the world and are classed in many countries as quarantine pests. Fruit flies have, however, no quarantine status in Austria because of the cold winters, making the IPCL a unique place to culture, maintain and carry out research on these important pests.

The IPCL plays a central role in a new CRP entitled Resolution of Cryptic Species Complexes of Tephritid Pests to Overcome Constraints to SIT Application and International Trade. The CRP was initiated in 2010 following increasing demands from Member States to resolve the uncertain taxonomic status of some fruit fly pests that exist as species complexes, i.e. fruit fly species that are morphologically similar and are not separate species but only geographical variants. This is of crucial importance and results in possible unjustified trade barriers for important commercial fruit and vegetable commodities.

FIG. 6. Anastrepha fraterculus (left) and Bactrocera dorsalis (right).

In addition, some fruit fly populations that are grouped within the same species display different biological and genetic traits, which have important practical and economic implications for the effective use of the SIT. The IPCL has initiated the establishment of various new colonies of fruit fly species that belong to the *Anastrepha fraterculus* and *Bactrocera dorsalis* complexes, in addition to several other colonies (Fig. 6). In December 2010, a total of 31 colonies of fruit flies were in culture at the IPCL, attracting researchers from a number of countries to carry out comparative studies of these major pest insects that are not possible in other parts of the world.

Anastrepha fraterculus *complex*

It is known that the South American fruit fly is composed of a complex of cryptic species comprising several morphotypes. The application of the SIT against this very important pest in South America poses problems when dealing with these reproductively isolated morphotypes that will not mate with adults of a different geographical region. When released, sterile males of the wrong morphotype will not mate with the female flies of the target area and the SIT will not work. Earlier work at the IPCL already showed that mating barriers exist between populations from Peru and Argentina.

Colonies of *A. fraterculus* originating from Tucuman (Argentina), and Vacaria and Pelotas (Brazil) were initiated at the IPCL. During field cage studies, sexually mature adults from these three populations mated at random and yielded offspring that was fully fertile. These results, combined with earlier data, seem to indicate that the area from Buenos Aires to São Paulo could be managed with one strain of *A. fraterculus.* Further work is planned with populations from Northern Brazil and Colombia.

Bactrocera dorsalis *complex*

Colonies were initiated at the IPCL of five species belonging to the *Bactrocera dorsalis* complex: *B. dorsalis*, *B. carambolae*, *B. philippinensis*, *B. invadens* and *B. papayae*. Initial field cage studies between *B. dorsalis* and *B. carambolae* indicated a relatively high level of mating isolation supporting their current taxonomic status. In contrast, mating studies between *B. papayae* and *B. dorsalis*, *B. papayae* and *B. philippinensis* and *B. dorsalis* and *B. philippinensis* revealed complete random mating.

Olive fly rearing

The olive fly *Bactrocera oleae* is the dominant pest of olives in the Mediterranean basin. It is highly invasive and has spread to California and Arizona in the USA and to Northern Mexico. For decades, farmers have been demanding alternatives to the use of insecticides, which has been the traditional way of controlling this important pest. In the past, the development of the SIT for this pest was hampered by difficulties with its rearing.

In 2010, significant progress was made with the further development of mass-rearing techniques for the olive fly. New production cages measuring 201 × 100 × 20.5 cm with a wax coated ovipositioning panel were seeded with 1.2 L of pupae. The maximum amount of eggs collected from such a cage was 91 mL, or 3.1 million eggs, over a period of three weeks. The trend in production efficiency continues to be upward, indicating that olive fly mass-rearing is becoming a reality. The technology is being transferred to Israel, where a pilot field project is ongoing.

Mosquitoes

For several years, various Member States have requested assistance with the development of the SIT package for disease transmitting mosquitoes. Initially, the research was focused on the malaria transmitting mosquito *Anopheles arabiensis*, but it was expanded in 2010 to *Aedes albopictus*, an important invasive species that serves as a vector for diseases such as dengue and chikungunya. Mosquito SIT is at its infancy and therefore much of the research effort goes into developing rearing techniques that will enable the production of large numbers of the target insect. Unlike the adults, mosquito eggs, larvae and pupae are all water based, and the efficient handling of large volumes of water remains one of the great challenges in a mosquito mass-rearing facility.

Work continued with the further development of a flat larval holding tray that has a large surface area of shallow water that mimics natural breeding sites. A prototype rack system with a capacity to hold 50 larval trays was designed and the effects of reduced light conditions

(the trays in the rack are only 3 cm apart) were assessed on development parameters. Water evaporation rates and water temperature in relation to the position of the tray in the rack were also assessed. As larvae development is not synchronized, and in view of the short pupal period, the developed young pupae have to be removed from the production line each day. A stainless steel, industrial version of the larvae–pupae separator was constructed and successfully tested. Finally, a new prototype adult holding cage was designed with two walls consisting entirely of netting. Ease of manipulation, egg oviposition, production rate, etc., are currently being evaluated.

Mosquito-borne diseases are transmitted by female mosquitoes only, as blood is a requirement for egg development. Male mosquitoes do not bite and can therefore be sterilized and released without increasing disease transmission rates. However, such SIT programmes require the complete removal of the female sex to avoid introducing potential vectors into the target area. An *An. arabiensis* GSS that is based on an insecticide resistant mutation was developed a few years ago. Treating larvae of this strain with dieldrin, a potent insecticide, kills the female but not the male mosquitoes. In order to increase the rearing efficiency, work has been conducted on assessing the feasibility of treating eggs with dieldrin. The effects of egg age, dieldrin concentration and treatment time have been investigated.

The radiation dose administered to the male sex is one of the quality reducing factors of released insects. The effect of gamma rays on egg hatch and survival of the male mosquitoes of the genetic sexing strain of *An. arabiensis* was therefore assessed for comparison with wild-type strains. The level of sterility induced in *Ae. albopictus* after exposure to X rays was similar to results obtained with gamma rays from a ^{60}Co and ^{137}Ce source. In field cages, mating competitiveness of male *Ae. albopictus* seemed age dependent, with older males being more competitive than young males. Irradiation of pupae and treating eggs of the GSS with dieldrin reduced the number of sperm as compared to untreated GSS and wild-type males. Whereas in untreated males the amount of sperm increased with age, the opposite was observed in irradiated males. This aspect is being further investigated.

CAPACITY BUILDING

In 2010, the IPCL hosted four cost-free experts: Mark Schutze (Australia — 9 months — mating studies of *Bactrocera dorsalis*), Adalecio Kovaleski (Brazil — 8 months — mating studies with *Anastrepha fraterculus*), and Guy Hallman and Scott Myers (USA — 2 and 3 weeks, respectively — post-harvest cold treatment of fruit flies). It also hosted two interns, Odessa Madakacherry (USA — 7 months — mosquitoes) and Arianna Puggioli (Italy — 2.5 months — mosquitoes), and five fellows funded by the Department of Technical Cooperation, namely Nwe Nwe Yin (Myanmar — 11 months — fruit flies), Henry Kariithi (Kenya — 2.5 months — tsetse), Mehrad Ahmadi (Islamic Republic of Iran — 5 months — fruit flies and tsetse), Rania Ahmed (Sudan — 2 months — mosquitoes) and Giselle Ouedraogo (Burkina Faso — 2.5 months — tsetse). In addition, the IPCL hosted Inamullah Khan from Pakistan for 2.5 months as a cost free fellow.

The IPCL also hosted five consultants: Gratian Mutika (Zimbabwe — 12 months — tsetse field cage studies), Idrissa Kabore (Burkina Faso — 12 months — tsetse irradiation and cold treatment studies), David Damiens (France — 3 months — mosquito sperm studies), Ihsan ul Haq (Pakistan — 10.5 months — fruit fly behaviour studies) and Fabrizio Balestrino (Italy — 2 months — mosquito mass rearing equipment development).

SERVICES

The IPCL continuously receives requests from collaborators in CRPs and in TC projects, and from universities and research institutes for the supply of biological material. In 2010, the IPCL supplied 96 790 tsetse fly pupae (*G. pallidipes, G. palpalis gambiensis and G. m. centralis*) to five research institutes in Slovakia, South Africa, the United Kingdom and the USA. In addition, the IPCL supplied 2400 pupae and 0.05 mL of eggs of the olive fly *Bactrocera oleae*, 3200 mL of VIENNA 8 (*Ceratitis capitata*) pupae, 400 mL of VIENNA 8 eggs, 400 pupae of melon fly *Bactrocera cucurbitae*, 13 600 pupae of South American fruit fly *Anastrepha fraterculus*, and 500 pupae of Mexican fruit fly *Anastrepha ludens*. In addition, the IPCL provided 11 kg of fruit fly diet. The fruit fly pupae were delivered to 14 research institutes in Argentina, Australia, Croatia, Czech Republic, Germany, Israel, Italy, Mauritius, the Netherlands, Singapore, Spain and the United Kingdom.

PUBLICATIONS

Abd-Alla, A.M.M., Kariithi, H., Parker, A.G ., Robinson, A.S.,. Kiflom, M., Bergoin, M. and Vreysen, M.J.B. (2010). Dynamics of the salivary gland hypertrophy virus in laboratory colonies of *Glossina pallidipes* (Diptera: Glossinidae). Virus Research 150: 103–110.

Abd-Alla, A.M.M., Boucias, D.G. and Bergoin, M. (2010). *Hytrosaviruses: Structure and Genomic Properties*. In: Asgari, S. and Johnson, K.N., (Eds), Insect Virology. Caister Academic Press, Norfolk, UK, pp. 103–121.

Alphey, L., Benedict, M.Q., Bellini, R., Clark, G.G., Dame, D.A., Service, M.W. and Dobson, S.L. (2010). Sterile-insect methods for control of mosquito-borne diseases: an analysis. Vector-Borne and Zoonotic Diseases 10(3): 295–311.

Balestrino, F., Medici, A., Candini, G., Carriere, M., Maccagnani, B., Calvitti, M., Maini, S. and Bellini, R. (2010). Gamma ray dosimetry and mating capacity studies in the laboratory on *Aedes albopictus* males. Journal of Medical Entomology 47(4): 581–591.

Balestrino, F., Soliban, S.M., Gilles, J., Oliva, C. and Benedict, M.Q. (2010). Ovipositional behaviour in the context of mass rearing of *Anopheles arabiensis*. Journal of the American Mosquito Control Association 26: 365–372.

Bellini, R., Albieri, A., Balestrino, F., Carrieri, M., Porretta, D., Urbanielli, S., Calvitti, M., Moretti, R. and Maini, S. (2010). Dispersal and survival of *Aedes albopictus* (Diptera: Culicidae) males in Italian urban areas and significance for sterile insect technique application. Journal of Medical Entomology 47(6): 1082–1091.

Benedict, M., Eckerstofer, M., Franz, G., Gaugitsch, H., Greiter, A., Heissenberg, A., Knols, B., Kumschick, S., Nentwig, W. and Rabistsch, W. (2010). Defining environmental risk assessment criteria for genetically modified insects to be placed on the EU market. CT/EFSA/GMO/2009/03, 1-200. Parma, EFSA.

Bouyer, J., Ravel, S., Guerrini, L., Dujardin, J.-P., Sidibe, I., Vreysen, M.J.B., Solano, P. and De Meeus, T. (2010). Population structure of *Glossina palpalis gambiensis* (Diptera: Glossinidae) between river basins in Burkina Faso: Consequences for area-wide integrated pest management. Infection, Genetics and Evolution 10: 321–328.

Bouyer, J., Seck, M.T., Sall, B., Ndiaye, E.Y., Guerrini, L. and Vreysen, M.J.B (2010). Stratified entomological sampling in preparation for an area-wide integrated pest management program: the example of *Glossina palpalis gambiensis* (Diptera: Glossinidae) in the Niayes of Senegal. Journal of Medical Entomology 47(4): 543–552.

Briceño, R.D., Wegrzynek, D., Chinea-Cano, E., Eberhard, W.G. and Santos Rolo, T. (2010). Movements and morphology under sexual selection: tsetse fly genitalia. Ethology Ecology & Evolution 22: 385–391.

Dominiak, B.C., Sundaralingam, S., Jiang, L., Jessup, A.J. and Nicol, H.I. (2010). Impact of marking dye, transport and irradiation on eclosion of mass produced Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). Plant Protection Quarterly 25(3): 141–143.

Feldmann, U. and Parker, A. (2010). The role of the sterile insect technique (SIT) in tsetse control: Using a pest to attack itself. Public Health Journal 21: 38–42.

Gabrieli, P., Falaguerra, A., Siciliano, P., Gomulski, L.M., Scolari, F., Zacharopoulou, A., Franz, G., Malacrida, A.R. and Gasperi, G. (2010). Sex and the single embryo: early development in the Mediterranean fruit fly, *Ceratitis capitata*. BMC Developmental Biology 10: 12.

Ul Haq, I., Mayr, L., Teal, P.E.A., Hendrichs, J., Robinson, A.S., Stauffer, C. and Hood-Nowotny, R. (2010). Total body nitrogen and total body carbon as indicators of body protein and body lipids in the melon fly *Bactrocera cucurbitae:* effects of methoprene, a juvenile hormone analogue, and of diet supplementation with hydrolysed yeast. Journal of Insect Physiology 56: 1807–1815.

Ul Haq, I., Cáceres, C., Hendrichs, J., Teal, P., Wornoayporn, V., Stauffer, C. and Robinson, A.S. (2010). Effects of the juvenile hormone analogue methoprene and dietary protein on male melon fly *Bactrocera cucurbitae* (Diptera: Tephritidae) mating success. Journal of Insect Physiology 56(11): 1503–1509.

Ul Haq, I., Cáceres, C., Hendrichs, J., Teal, P., Wornoayporn, V., Stauffer, C. and Robinson, A.S. (2010). Methoprene modulates the effect of diet on male melon fly, *Bactrocera cucurbitae,* performance at mating aggregations. Entomologia Experimentalis et Applicata 136(1): 21–30.

Kariithi, H.M., Ince, A.I., Boerren, S., Vervoort, J., Bergoin, M., Van Oers, M.M., Abd-Alla, A.M.M. and Vlak, J.M. (2010). Proteomic analysis of *Glossina pallidipes* salivary gland hypertrophy virus virions for immune intervention in tsetse fly colonies. Journal of General Virology 91(12): 3065–3074.

Mastrangelo, T., Parker, A.G., Jessup, A., Pereira, R., Orozco-Dávila, D., Islam, A., Dammalage, T. and Walder, J.M.M (2010). A new generation of X ray irradiators for insect sterilization. Journal of Economic Entomology 103: 85–94.

Sasanya, J.J., Abd-Alla, A.M.M., Parker, A.G. and Cannavan, A. (2010). Analysis of the antiviral drugs acyclovir and valacyclovir-hydrochloride in tsetse flies (*Glossina pallidipes*) using LC-MSMS. Journal of Chromatography B 878: 2384–2390.

Seck, M.T., Bouyer, J., Sall, B., Bengaly, Z. and Vreysen, M.J.B. (2010). The prevalence of African animal trypanosomoses and tsetse presence in western Senegal. Parasite 17(3): 257–265.

Simmons, G.S., Carpenter, J.E., Suckling, M., Addison, M., Dyck, A. and Vreysen, M.J.B. (2010). Improved quality management to enhance the efficacy of the sterile insect technique for lepidopteran pests. Journal of Applied Entomology 134: 261–273.

Solano, P., Kaba, D., Ravel, S., Dyer, N.A., Sall, B., Vreysen, M.J.B., Seck, M.T., Darbyshir, H., Gardes, L., Donnelly, M.J., Meeus, T.D. and Bouyer, J. (2010). Population genetics as a tool to select tsetse control strategies: suppression or eradication of *Glossina palpalis gambiensis* in the Niayes of Senegal. PLoS Neglected Tropical Diseases 4: 5.

Taret, G., Sevilla, M., Wornoayporn, V., Islam, A., Ahmad, S., Cáceres, C., Robinson, A.S. and Vreysen, M.J.B. (2010). Mating compatibility among populations of codling moth *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae) from different geographic origins. Journal of Applied Entomology 134: 207–215.

Vreysen, M.J.B. and Robinson, A.S. (Eds) (2010). Proceedings of an FAO/IAEA coordinated research project on improvement of codling moth SIT to facilitate expansion of field application. Journal of Applied Entomology 134(3): 163.

Vreysen, M.J.B., and Robinson, A.S. (2010) Ionising radiation and area-wide management of insect pests to promote sustainable agriculture. A review. Agronomy for Sustainable Development. doi: 10.1051/agro/2010009.

Vreysen, M.J.B., Carpenter, J.E. and Marec, F. (2010). Improvement of the sterile insect technique for codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera Tortricidae) to facilitate expansion of field application. Journal of Applied Entomology 134(3): 165–181.

Zepeda-Cisneros, C.S., Meza, J.S., Galvez, S., Ibañez, J. and Robinson, A.S. (2010). Inheritance and linkage studies on eye colour mutations in *Anastrepha ludens* (Diptera: Tephritidae). Annals of the Entomological Society of America 103(1): 96–99.

PLANT BREEDING AND GENETICS LABORATORY

EXECUTIVE SUMMARY

The global need for sustainable intensification of crop production is greater than ever before. Pressures from population growth, climate change and variability, and a shift to meat based diets that drive crop production for animal feed continue to increase. The challenge is made greater by unpredictable events such as natural disasters and political unrest that can threaten food security. A multi-faceted approach is required to meet the demand. This includes enhancing the efficiency of plant breeding so that new crop varieties with higher yields, better nutrition and increased resistance to diseases and climatic variability can be produced and made available to all countries. Through its three major mechanisms of technology development and adaptation, capacity building, and the provision of technical services, the Plant Breeding and Genetics Laboratory (PBGL), partnered with the Plant Breeding and Genetics Section (PBGS) in the Sustainable Intensification of Crop Production Systems (SICPS) sub-programme of the Joint FAO-IAEA Division, supports Member States in their efforts to improve agricultural productivity.

Efforts in the area of technology development and adaptation are aimed at developing efficient strategies for the induction, recovery and characterization of mutation events that create novel and useful traits for plant breeders, and those involved in pre-breeding applications. This work covers the broad categories of molecular biology, reverse genetics, phenotyping and cell and tissue biology. We focus on three major commodity crops: *Musa* (banana and plantain), *Manihot esculenta* (cassava), and *Oryza sativa* (rice). These represent staples for billions of people in the developing world. Banana and cassava serve as models for vegetatively propagated species, and rice is a model for sexually reproducing species. The PBGL is working to enhance the process of mutation induction, to increase the efficiency of dissolving chimeric sectors and selection of plants with improved traits, and to adapt the reverse genetics strategy known as TILLING so that it is suitable for a large number of crops. These approaches represent technology packages. Modular in nature, such packages can be combined to create a pipeline to improve the success and speed of breeding. It is envisioned that a suite of technology packages can be developed that can be combined to address a broad range of constraints. The PBGL is collaborating with counterparts in an IAEA sponsored CRP to further establish such technology pipelines. To facilitate technology transfer to Member States, the PBGL is developing protocols, guidelines and kits for low cost assays to induce, discover and characterize plants with advantageous mutations from treatment with gamma and X rays.

Human capacity building through directed training took the form of supporting multi-month research fellowships at the PBGL, training interns and hosting scientific visitors. In 2010, 16 research fellows from 13 countries, two interns from two countries and eight scientific visitors from five countries were trained. The laboratory also hosted a two week Interregional Training Course where participants learned techniques for improving disease and climate resistance in wheat and barley. The PBGL continuously maintains expertise and capacity to work on a variety of crops of interest to Member States. Over the course of the year, research

fellows at the PBGL were trained on mutation based methods and adapted these methods for diverse crops including banana, mango, rice, sorghum, wheat, barley, fenugreek, garlic, common bean, jatropha and sesame.

Efforts to support the capacity of Member States to use induced mutations to develop superior crop varieties also included the provision of technical services for mutation induction, ploidy and genetic analysis. Twenty-five requests from 17 Member States were processed by the PBGL, many tied to research projects of visiting fellows.

Presentations of the PBGL's adaptive R&D activities were given at five international conferences, and publications included peer reviewed research and review articles, four book chapters and published conference abstracts.

STAFF

Name	Title	Email	Extension
Mba, Chikelu[a]	Unit Head		
Till, Bradley John[b]	Plant Biotechnologist	B.Till@iaea.org	28260
Afza, Rownak[a]	Consultant		
Matijevic, Mirta	Technician	M.Matijevic@iaea.org	28317
Jankowicz-Cieslak, Joanna Beata	Technician	J.Jankowicz@iaea.org	28275
Berthold, Guenter	Technician	G.Berthold@iaea.org	28418
Draganitsch, Andreas	Technician	A.Draganitsch@iaea.org	28418
Bado, Souleymane	Technician	S.Bado@iaea.org	28208
Huynh, Owen Anthony	Technician	O.Huynh@iaea.org	28279
Seballos, Gilbert	Laboratory Attendant	G.Seballos@iaea.org	28278
Lorenz, Anne	Secretary	A.Lorenz@iaea.org	28274
Swoboda, Lisa	Secretary	E.Swoboda@iaea.org	28362

[a] Left the PBGL in March 2010.
[b] Acting Unit Head from March 2010.

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The broad goals of adaptive R&D activities at the PBGL are to strengthen the capacity of Member States to improve the efficiency of induction, monitoring, evaluation and selection of mutations that enhance crop productivity. Important areas for improvement include biotic and abiotic stresses and quality traits. Outputs of adaptive R&D activities include increased knowledge about the mechanisms of mutation induction and inheritance, and gene function, and also the development and adaptation of protocols for and guidelines on the efficient use

of induced mutations to breed superior crops. A variety of protocols and guidelines updated in 2010 as well as the PBGL Laboratory Manual can be found on http://mvgs.iaea.org/LaboratoryProtocals.aspx (see "Protocols, Guidelines and Information" for additions in 2010).

Mutation induction to develop superior crops

The use of induced mutations to generate heritable genetic changes in crops dates back to the late 1920s. It is simply a method to increase the rate of mutation, a process that occurs naturally on Earth and is a major driver of evolution. The great biodiversity observable today is a testament to the power of mutations to generate a wide range of forms and functions in biological systems.

The success of using induced mutations to develop improved crops can be measured in billions of dollars annually. While inducing mutations in plants by treating seed or tissue with ionizing radiation is decades old, new technologies promise to unlock a hidden potential and allow more targeted and efficient mutation breeding strategies. In this context, a core activity of the PBGL is to improve the process of mutation induction so that densities and spectrums of induced alleles can be optimized to increase the probability of creating useful traits while reducing the effects of unwanted background mutations. Projects in 2010 included studies surrounding the effect of different doses of gamma and X rays on banana, cassava and rice. Gamma ray irradiation produced from a ^{60}Co source has been perhaps the most widely used and successful method for inducing mutations for breeding. Major emerging bottlenecks, however, are limitations in the transportation and installation of ^{60}Co sources. This means that Member States may not have access to facilities to produce the raw materials for their breeding programmes. While less frequently applied, X rays are an attractive alternative due to their ubiquity in most countries in the form of medical devices. Historically, X rays were the first form of ionizing radiation used to alter a plant's genetic makeup. Therefore, the PBGL focused its R&D activities on developing protocols and guidelines to enable Member States to have the same breeding successes with X rays as have been previously achieved with gamma irradiation. The first step in generating these guidelines is the preparation of a mutant population that has been propagated to remove chimeric sectors arising from the mutation process, and that is at the appropriate generation to ensure statistically significant phenotypic and genotypic comparisons.

The major achievement in 2010 was the development of mutagenized populations suitable for the comparative evaluation of different mutagens and dosages. In rice, a total of five upland and irrigated varieties of the two major subspecies (*indica* and *japonica*) were treated with different dosages of gamma and X rays from 75 to 600 Gy. Growth and fecundity of the M_1 population were measured under the climatic conditions of the Seibersdorf greenhouse and one variety (upland *japonica*) was selected as the best for downstream evaluations. Around 4600 M_2 plants were then prepared from self-fertilization of the M_1. This provides the base material for phenotypic and genotypic studies begun in 2010 and continuing in the future. Knowledge and protocols being developed by the PBGL for rice can easily be applied to the other grains such as maize, sorghum, barley and wheat.

The triploid banana variety of the genus *Musa* that is commonly eaten, being sterile and parthenocarpic, is a model for obligate vegetatively propagated crops. Here, the ideal mutagen would induce changes that are either dominant in nature, or uncover hemizygous allele states created through the accumulation of natural deleterious mutations and successive rounds of mitotic propagation. Unlike seed propagated crops, major novel bottlenecks exist for the genetic improvement of obligate vegetatively propagated crops like bananas. With no means to bulk plants through sexual fertilization, and no possibility to remove chimeric sectors through meiosis, tissue culture protocols and population management strategies must be developed for practical use of induced mutations to create useful traits. The need for such improved methods is exemplified by the major threats to *Musa* production such as fungal pathogens and drought that can destroy entire plantations. This is a food security threat, as 87% of production is consumed locally in developing Member States. There are few examples of vegetatively propagated mutant varieties registered in the IAEA database compared to seed propagated crops. One possible reason for this is the increased genetic bottlenecks present when plants are propagated vegetatively, such as the challenges to make mutations homohistont in material treated with ionizing radiation.

Thus, improved methods aimed at overcoming limitations may allow many more vegetatively propagated mutant varieties to be developed and released. Major achievements in vegetatively propagated species were the development of streamlined methods for the production of mutagenized plants. In banana, 4000 clonally propagated plants were prepared and mutagenized. Samples were collected and evaluated both phenotypically and genotypically. As described below, information from the analyses is being used to produce protocols and guidelines for rapid production of homohistont material. Cassava, a major staple for 500 million people in the tropics and subtropics, is a model for facultative vegetative propagation where sexual reproduction is difficult but not impossible. In cassava, a strategy was devised to track diplontic selection of meristematic cells of the primary meristem. For this, base material of 3000 gamma irradiated plants was created and sent to counterparts for field evaluations.

Monitoring, evaluating and selecting mutated crops for improved characteristics

Traditional phenotyping is the act of monitoring observable differences in crop traits arising due to differences in growth environment, natural genetic diversity or diversity induced through mutation. This remains the major tool for breeders to evaluate and select plants for incorporation or maintenance in breeding programmes. The PBGL is engaged in phenotyping activities in all of its core crops to better evaluate the effects of different mutagens and dosages in plants in order to optimize and improve the efficiency of the induced mutation process. One major achievement was the characterization of the M_2 mutant rice plants produced in the PBGL by treatment of M_0 material with gamma and X rays. Measurements of morphological parameters, growth rate, chlorophyll content, cytological measurements of microspores viability, seed morphology and flowering time were recorded. Based on preliminary comparative analysis, guidelines are being drafted on X ray dosages that correlate with previously optimized gamma dosages. This should allow similar breeding results to be achieved by Member States using X rays for irradiation. Comprehensive statistical analyses are ongoing, and a protocol for publication is being prepared.

Abiotic stresses are a major cause of crop yield reduction. To evaluate the effects of, and optimize mutagenesis for improvement of resistance to, abiotic stress in cereals, the PBGL adapted protocols for rapid salinity screening for both soil and liquid growth cultures. While liquid culture screening has been well established, one goal is to produce reliable soil based methods that are lower in cost and can be more easily transferred to developing Member States. This multi-year project included six rice genotypes: two tolerant, two moderate and two susceptible varieties. A major achievement in 2010 was the collection and evaluation of phenotypic data in salt stressed plants. Visual growth effects were monitored in both soil and liquid growth conditions. Along with biomass, more sensitive measurements using carbon isotope discrimination and mineral analysis using spectrophotometric and fluorometric methods were applied. Analysis of the data shows correlations between growth performance in higher saline environments and tissue specific accumulation of chloride and sodium ions. Understanding how the different rice varieties are able to cope with increasing saline will allow gene targeted approaches to use mutations to improve tolerance in susceptible varieties. The salinity screening methods represent a technology package developed by the PBGL which is being improved through the addition of guidelines on how to best evaluate plants for rapid selection of resistant mutants.

The methods have been adapted by the PBGL for wheat and barley varieties, and this was disseminated to 25 scientists from 18 Member States during the Interregional Training Course on Mutation Induction and Breeding for Cereal (Wheat and Barley) Rust Resistance and Climate Hardening/Harsh Environment Adaptation Part I.

For optimization of abiotic stress screening in vegetatively propagated crops, a population of ~1500 mutagenized banana plants has been developed through tissue culture mutagenesis and meristematic isolation and cutting to dissolve chimeric sectors. Plants were taken to the M_1V_6 generation and subjected to molecular assays for mutation discovery (see next section). Genes that are hypothesized to be important for drought resistance were screened, and mutant candidates were recovered that were then subjected to drought profiling. For drought profiling, 36 *Musa* accessions comprising diploids, polyploids and hybrids of the *acumiata* and *balbisiana* genome types, along with selected plants having point mutations in the putative drought resistance genes, were grown to the four leaf stage in the greenhouse and then subjected to increasing drought stress measured as field water capacity. At each of the selected four drought stages, a variety of measurements were recorded including transpiration, CO_2 concentration, stomatal conductance, net photosynthesis, activity of the photosystem II and carbon isotope discrimination. Preliminary evaluations suggest no clear correlation between genome type or ploidy level and response to stress. Because fertile diploid cultivars are used in breeding programmes to recapitulate seedless triploids, careful evaluation of diploids is therefore recommended prior to selection of cultivars for traditional and mutation aided breeding projects aimed at improving drought resistance. This work was done in collaboration with the University of Krakow (see 'External collaborations and partnerships', below). The PBGL has further developed a guideline for *Musa* drought screening that uses 80% and 35% field water capacity at key time points in drought screening for selecting improved mutant plants. Another major achievement of this work is the development of drought profiling data for the core set of *Musa* accessions used by scientists around the world. Other researchers

have used the same core set of samples for different analyses, and the combination of data represents a rich resource for the banana community.

Biotechnologies for measuring, selecting and optimizing induced mutation events

Modern biotechnologies provide several avenues for increasing the efficiency of agricultural improvement. The use of molecular markers, DNA polymorphisms linked to or causing trait variation, provides a means of selecting elite material in the absence of phenotypic selections that are labour intensive, subject to variability due to environmental growth conditions, and sometimes impractical (for example, selection of plants with enhanced resistance to a fungal pathogen would require treatment of plants with the pathogen that could lead to plant loss; selections based on molecular markers obviate the need to do this). In addition to selection based on linkages of genetic variability to a gene controlling a specific trait, biotechnologies can also be used for functional genomics applications and to target mutations to specific genes. For example, the PBGL has developed an Ecotilling platform for diverse *Musa* ecotypes. A major achievement was the development of a protocol for the co-discovery of heterozygous nucleotide polymorphisms in homologous sequences in hybrids and polyploids. This allows rapid differentiation between diploid, triploid and hybrid varieties. Thus, the method may serve as a replacement for the more laborious and time consuming flow cytometry technique that requires specialized equipment and expertise. This work was disseminated to the community through a peer reviewed publication in 2010. To facilitate the use of these tools in laboratories in developing Member States, low cost assays were developed, validated and transferred to counterparts in Mauritius for evaluation of local banana varieties and mutants.

Mauritius served as a test of our technology transfer approach, as validated materials are temperature labile and appropriate shipping and post-shipping handling methodologies needed to be tested, along with the performance of materials and accompanying protocols, in an off-site laboratory The low cost method employs standard equipment and procedures such as agarose gel electrophoresis and PCR with unlabelled primers that are available in most laboratories engaged in molecular biology assays. At the core of this method is a kit designed by the PBGL to assist Member States in mutation discovery (see below). A current barrier for *Musa* is the high level of heterozygosity that hinders the use of enzymatic mismatch cleavage assays for the recovery of homozygous allele differences that can be used in phylogenetic studies. Work is ongoing to develop a doubled-haploid based method for efficient recovery of both heterozygous and homozygous alleles for more detailed analysis of *Musa* populations.

With the aim of enhancing the mutation induction process in vegetatively propagated species, enzymatic mutation discovery methods were applied to 768 mutagenized bananas in the M_1V_6 generation for the discovery and tracking of mutation events in approximately 15 gene targets. The recovery of over 20 induced mutation events was validated through Sanger sequencing. Importantly, the population was structured such that the inheritance of mutation events could be monitored in sibling clones, allowing for an estimation of the vegetative cycle when chimeric sectors were genotypically dissolved in meristematic tissues. This work is ongoing. The PBGL is developing models for how quickly plants are made genotypically homogeneous through diplontic selection and tissue culture manipulations. The expectation is that extensive labour investment in tissue culture can be greatly reduced. Furthermore, true inheritance

of recovered mutations from M_1V_6 to M_1V_9 has been validated. Using the reverse genetics strategy TILLING (for Targeting Induced Local Lesions IN Genomes), mutations have been targeted to, and recovered in, genes hypothesized to be important for drought response. The power of reverse genetics is that interesting mutants can be selected from a large population and only those plants need to be subjected to laborious phenotypic assays. In this case, only 2 of 768 plants were selected as candidates because they harboured non-synonymous mutations and all other induced mutations were predicted to be silent. These plants were subjected to the drought profiling experiments described above. The major achievements of this work are the establishment of TILLING in a triploid cultivar, explicit knowledge of the density and spectrum of induced mutations and a model for dissolution of chimeric sectors. Combined, these scientific results provide a framework of guidelines for the effective use of induced mutations and reverse genetics in a variety of vegetatively propagated species including potato and citrus. This has been disseminated at international scientific conferences.

Parallel to the work in banana, a population of over 3000 gamma irradiated cassava plantlets have been produced and subjected to TILLING assays in 7 starch biosynthetic pathway genes using enzymatic mismatch cleavage assays. Importantly, cassava grows in a nodal fashion and this is being exploited to evaluate chimeric dissolution from the primary meristem. The work to date has established that the level of extant heterozygosity in many varieties is sufficiently low, and that the spontaneous mutations are rare. This suggests that TILLING can be successfully applied to cassava. Less is known, however, about the optimal mutation dosages. The work of the PBGL suggests a very low mutation density at the dosages examined. However, due to an ascertainment bias in the enzymatic methods used to discover mutations, large deletions may have gone unnoticed. Future work includes evaluation of alternative technologies for discovery of a wider spectrum of allele types.

CAPACITY BUILDING

In 2010, 51 research scientists were trained by the PBGL through the mechanisms of TC research fellowships, internships, scientific visits and an interregional training course in 2010. Trainees came from: Algeria, Argentina, China, Egypt, Eritrea, Ethiopia, India, the Islamic Republic of Iran, Iraq, Jordan, Kenya, Lebanon, Madagascar, Mauritius, Pakistan, the Philippines, Poland, Saudi Arabia, Senegal, South Africa, the Syrian Arab Republic, Tunisia, Turkey, Uganda, Yemen and Zambia.

In addition to the training of fellows in the low cost polymorphism technology package described above, fellowship training in the PBGL, under the guidance of laboratory staff, was done to adapt and optimize the induction of mutations in wheat, sorghum, jatropha, barley, common bean, groundnut, carrot, cotton, date palm, sesame, garlic and lupinus in support of Member States' requests for assistance in using nuclear techniques to improve these crops. For example, in vitro methods such as microspore culture were adapted to speed up the dissolution of chimeric tissues arising from mutagenesis and to generate instantly homozygous plants. This protocol, developed for lupinus, is being validated in the PBGL for use in rice. Such methods can reduce the breeding time by years.

The Interregional Training Course on Mutation Induction and Breeding for Cereal (Wheat and Barley) Rust Resistance and Climate Hardening/Harsh Environment Adaptation Part I was held for two weeks in the PBGL. Twenty-five participants from 18 Member States attended to learn efficient strategies for using induced mutations for crop improvement. Fungal pathogens that cause cereal rusts can destroy crops and such diseases are now global threats to world agriculture as climatic variations are causing pathogens to spread across borders. Climatic changes are also driving increased pressure on water availability and other factors that further threaten crop production. The use of induced mutations for crop improvement marks a main avenue to combat such problems. The course brought together PBGL staff and five external lecturers to transfer knowledge to researchers in Member States looking for safe, effective and efficient methods to support sustainable food security. These trainees will go on to train others thus broadening the impact of capacity building.

Fellows

Name	Country	Duration	Training topics
Al-Kaabi, Ekhlas	Iraq	1 month	Group fellowship on: Mutation induction, seed, in vitro, applications of plant tissue culture, morphogenesis, organogenesis, somatic embryogenesis, micropropagation, DH, microspores/ anthers culture, chromosome doubling, regeneration, hardening, mutant characterization molecular techniques, cytological techniques, flow-cytometry, (molecular) cytogenetics, in vitro screening, salt tolerance, and disease resistance
Alhajaj, Nawal	Jordan	1 month	
El Bitar, Ahmad	Lebanon	1 month	
Alsalman, Abdullah	Saudi Arabia	1 month	
Alzahrani, Saad	Saudi Arabia	1 month	
Saleh, Basel	Syrian Arab Republic	1 month	
Salem, Ali	Yemen	1 month	
Alsalehi, Abdulwahab	Yemen	1 month	
Razafinirina, Lydia	Madagascar	4 months	Induced mutations for improvement of rice; somatic embryogenesis for Malagasy rice varieties; ploidy analysis
Dussoruth, Babita	Mauritius	3 months	Induced mutations for improvement of banana; ploidy analysis; genetic diversity study of different accessions of banana varieties from Mauritius
Muimui, Kenedy Katazo	Zambia	4 months	Induced mutations for improvement of beans; characterization of putative mutants using molecular techniques

Name	Country	Duration	Training topics
Diedhiou,Papa Mdaiallacke	Senegal	3 months	Induced mutations for improvement of Jatropha and peanut; somatic embryogenesis; characterization of putative mutants using molecular techniques
Gherezghiher Tecle, Efrem	Eritrea	6 months	Induced mutations for improvement of sorghum; characterization of Eritrean accessions; mineral analysis
Alforque, Marilyn	Philippines	3 months	Mutation induction in vegetatively propagated plants; phenotypic characterization of mutation population at M1V10; development of mutant rice
Tiliouine, Wahiba	Algeria	3 months	Molecular biology techniques for the genotyping of putative barley mutants
Alfaoury, Hussam	Syrian Arab Republic	5 months	Genetic diversity study of different accessions of Daucus, cotton and date palm varieties from the Syrian Arab Republic; TILLING and Ecotilling for characterization of Syrian Daucus and cotton germplasm, and putative sesame and garlic mutants

Scientific Visitors

Gueye, Tala	Senegal	5 days	Induced mutations in crop improvement and related biotechnologies
Ndoye, Khadidiaton Ndir	Senegal	5 days	
Kaimoyo, Evans	Zambia	5 days	
Munyinda, Kalaluka	Zambia	10 days	
Muharram, Ismail	Yemen	5 days	
Berhane Ghebremariam, Tsegay	Eritrea	5 days	
Maghuly, Fatemeh	Austria	1 day	
Laimer, Margit	Austria	1 day	

Interns

Froestl, Federico	Argentina	1 month	Screening cassava mutagenic population developed for TILLING technology adaptation for starch quality
Kozak, Kamila	Poland	4 months	Cytological and phenotypical analyses of M2 rice plants; technology transfer to the *Lupinus* spp.

SERVICES

Services represent core technology packages to enable Member States to use induced mutations for breeding. Work is carried out at the PBGL facility, and information and materials are provided to Member States. Information on the services is disseminated to Member States via our web site (http://mvgs.iaea.org/LaboratoryProtocals.aspx) and is described at training courses and international conferences, and to fellows, interns and visiting scientists to the PBGL. The PBGL follows the International Treaty on Plant Genetic Resources for Food and Agriculture and the associated Standard Material Transfer Agreement that provides an efficient environment for Member States to transfer and share plant genetic materials.

In 2010, service requests came from independent research institutes and universities; some are involved in TC projects or CRPs, research fellows working at the PBGL and IAEA colleagues. Three services provided by the PBGL are mutagenesis, genotyping and flow cytometry. Web based on-line request forms were created in 2010. For mutagenesis, the ^{60}Co source of gamma rays and RS-2400 irradiator source of X rays, both of which can be found at the IAEA's Laboratories at Seibersdorf, were used to irradiate plant propagules of researchers from Member States. A total of 25 requests from 17 Member States and 2 international organizations were processed. The radiation sensitivity test was carried out for 12 crop species covering 38 varieties.

Genotyping, or the characterization and tracking of different genetic changes in plants, is an efficient method to characterize and select mutant plants harbouring beneficial changes. Such marker assisted approaches can reduce laborious and expensive field based monitoring and selection of mutant plant and save years in breeding time. Genotyping services in 2010 included AFLP, SSR and sequencing analysis of mutant varieties and DNA sequencing of identified mutations. This work included analysis of samples from internal R&D projects, research fellowship projects (TC projects), samples from colleagues in the Animal Production and Health Laboratory of the Joint FAO/IAEA Programme, and external requests from Member States (Algeria, Eritrea, Ghana, Senegal, South Africa, Syrian Arab Republic and Zambia). A total of 10 624 samples were analysed in 2010.

In addition, one request for flow cytometry was received from Mauritius. This method is used to rapidly characterize chromosome content in mutants and accessions, and for selecting plants for breeding and improvement schemes. In total, 10 banana samples from Mauritius were screened to determine ploidy levels. Diploid varieties are used in traditional breeding

and the edible triploid varieties are excellent targets for mutation based improvement. Proper characterization of varieties is therefore crucial for effective improvement strategies.

Irradiation services

Member State	Crop species
Bulgaria	Wheat
Eritrea	Sorghum
Germany	Ornamental plantlets (Sunsatia cranberry and Sunsatia lemon)
Jordan	Wheat and barley
Kenya	Barley
The Former Yugoslav Republic of Macedonia	Wheat
Madagascar	Rice (seeds and calli)
Philippines (IRRI)	Rice
Poland	Barley and lupins
Senegal	*Jatropha curcas* and groundnut
Spain	Caper spurge (*Euphorbia lathyris)* and Calla lily *(Zantedeschia aethiopica)*
Switzerland	Tobacco
United Republic of Tanzania	Rice
Turkey	Sesame
United Kingdom	Primula *(Primula vulgaris)* and wheat
Yemen	Wheat, lentil, barley, fenugreek and garlic
Zambia	Common bean

Kits developed and supplied to Member States

The TILLING protocol was adapted and developed into a low cost agarose based TILLING method for the discovery of induced mutations and natural polymorphisms to support molecular marker based approaches and TILLING and Ecotilling applications. This represents a technology package developed by the PBGL that is being delivered to Member States. Protocols are provided via our web site (http://mvgs.iaea.org/LaboratoryProtocals.aspx/) and described to visiting scientists at training courses, and also presented in seminars and international conferences. Research fellows at the PBGL have the opportunity for hands-on training on the protocols and the use of a kit containing all materials for protocol optimizations

including positive control samples, enzyme and buffer, to allow, for tailored optimizations for their target crops. In 2010, this kit was distributed to five laboratories in Brazil, India, Pakistan, Mauritius and the United States of America.

Protocols, Guidelines and Information released in 2010

on http://mvgs.iaea.org/LaboratoryProtocals.aspx

Title	Document type
Training Manual: Characterization of mutant germplasm	The PBGL's main training manual used in training courses
Mutation Induction for Breeding 101	Presentation on the basics of mutation breeding
Radiation Sensitivity Tables	Guidelines on chronic and acute dosages of radiation for a variety of different crop species
Instructions for using mutagenesis services	Guidelines on how to use the PBGL irradiation service
Mutagenesis Service Request Form, version 1.1	Request form for mutagenesis services
SMTA for Mutagenesis Services	Standard Material Transfer Agreement for mutagenesis service requests
Genotyping Services Cover Letter, version 2.1	Guidelines on how to use the PBGL genotyping services
Genotyping Services Request Form, version 2.2	Request forms for genotyping services
SMTA for Genotyping Services	Standard Material Transfer Agreement for genotyping requests
Positive control for mutation discovery using agarose gels, version 2.4	Protocol for mutation discovery using crude enzyme extracts and agarose gel electrophoresis
Positive control for mutation discovery using LI-COR and agarose gels, version 2.5	Protocol for mutation discovery using crude enzyme extracts and fluorescence detection and denaturing polyacrylamide gel electrophoresis
User Feedback form, mutation discovery positive control kit, version 1.1	Feedback form for mutation discovery kit

PUBLICATIONS

Tadele Z., Mba C. and Till B.J. (2010). TILLING for mutations in model plants and crops. In: Jain, S.M. and Brar, D.S. (Eds), Molecular Techniques in Crop Improvement, 2nd edition, Springer, the Netherlands, pp. 307–332.

Till, B.J. (2010). Mutation detection in plants by enzymatic mismatch cleavage. In: Meksem, K. and Kahl, G. (Eds), The Handbook of Plant Mutation Screening: Mining of Natural and Induced Alleles, Wiley-Blackwell-VHC, Germany, pp. 131–148.

Till, B.J., Jankowicz-Cieslak, J., Sagi, L., Huynh, O.A., Utsushi, H., Sweenen, R., Terauchi, R. and Mba, C. (2010). Discovery of nucleotide polymorphisms in the *Musa* gene pool by Ecotilling. Theoretical and Applied Genetics, 121(7): 1381–1389.

Wang, T., Uauy, C., Till, B.J. and Liu, C.M. (2010). TILLING and Associated Technologies. *Journal of Integrative Plant Biology,* 52(11): 1027–1030.

Jain, S.H., Till, B.J., Suprasanna, P. and Roux, N. (2010). Mutations and Cultivar Development in Banana. In: Pillay, M. and Tenkouano, A. (Eds), *Banana breeding: constraints and progress*; CRC Press, pp. 203–218.

Mba, C., Afza, R., Bado, S. and Jain, S.M. (2010). Induced Mutagenesis in Plants Using Physical and Chemical Agents. In: Davey, M.R. and Anthony, P (Eds), *Plant Cell Culture: Essential Methods*. DOI: 10.1002/9780470686522.ch7.

Lokko, Y., Mba, C., Spencer, M., Till, B.J. and Lagoda, P. (in press). Nanotechnology and Synthetic Biology: Potential in Crop Improvement. Intl. J. of Food, Agriculture and Environment.

Cooper, J.L., Henikoff, S., Comai, L. and Till B.J. (in press). TILLING and Ecotilling for Rice. In: Walker, J.M. (ed), Methods in Molecular Biology: Rice Protocols, Humana Press.

Till, B.J., Jankowicz-Cieslak, J., Huynh, O., Bado, S. and Matijevic, M. (2010). Reverse-genetic strategies for vegetatively propagated food security crops (Abstract). BBSRC China-UK workshop on TILLING and associated technologies, The Botany Institute, Chinese Academy of Sciences, Beijing, China, April 2010.

Till, B.J., Bado, S., Huynh, O.A., Matijevic, M. and Jankowicz-Cieslak, J. (2010). Reverse genetic strategies for vegetatively propagated crops (Abstract). Primer Congreso Peruano de Mejoramiento Genetico de Plantas Y Biotecnologia Agricola, La Molina, Peru, May 2010.

Maghuly, F., Jankowicz-Cieslak, J., Till, B., Calari, A., Ramkat, R. and Laimer, M. (2010). Biotechnological approaches to the production of elite plant material of Jatropha curcas (Abstract). In: International Association for Plant Biotechnology (IAPB), Sustainability through agricultural biotechnology: food, biomaterials, energy, and environment. IAPB Congress, St. Louis, Missouri, USA, June 2010.

Till, B.J., Jankowicz-Cieslak, J., Huynh, O., Bado, S. and Matijevic, M. (2010). Induction, maintenance and recovery of mutations in vegetatively propagated plants (Abstract). Plant Biodiversity and Food Diversification Symposium, Cluj-Napoca, Romania, September 2010.

EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
International Center for Tropical Agriculture (CIAT), Cali, Colombia	Induction and detection of mutation events in South American cassava lines for enhanced productivity and competitiveness through value addition
International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria	Induction and detection of mutation events in African cassava lines for enhanced productivity and competitiveness through value addition
International Rice Research Institute (IRRI), Manila, Philippines	Induced mutations in rice for tolerance to abiotic stresses (including salinity)
International Network for the Improvement of Banana and Plantains (INIBAP), Bioversity International, Montpellier, France	Induced mutations in *Musa* for tolerance to biotic stresses and development and deployment of genomics tolls for the crop
Austrian Institute of Technology, Health & Environment Department, (Silvia Fluch, Kornel Burg) Tulln, Austria	Gene expression profiling in drought stages
University of Agriculture, Department of Plant Physiology, (Marcin Rapacz) Krakow, Poland	Banana phenotyping for drought tolerance
University of Natural Resources and Life Sciences, Department of Biotechnology, (Theresa Scharl) Vienna, Austria	
University of Natural Resources and Life Sciences, Department of Biotechnology, (Margit Laimer, Fatemeh Maghuly) Vienna, Austria	Induced and natural mutations detection in understudied crops
Poznań University of Life Sciences, Department of Biometric, (Jan Bocianowski) Poznan, Poland	Statistical data evaluation of morphological characteristics measured in the various, mutated rice accessions (M1 and M2)
Laboratory of Tropical Crop Improvement, Katholieke Universiteit Leuven, Belgium	Germplasm characterization of *Musa* and TILLING for genes hypothesized to be important in drought response

SOIL AND WATER MANAGEMENT & CROP NUTRITION

EXECUTIVE SUMMARY

During 2010, the Soil and Water Management & Crop Nutrition Laboratory (SWMCNL) adapted and validated fallout radionuclides (FRN) based methodologies (^{137}Cs, ^{210}Pb and ^{7}Be) interfaced with a geographic information system (GIS) to estimate soil redistribution rates and inventories at field to catchment scales, and evaluated the efficiency of soil conservation measures under a wide range of agro-ecological conditions, taking into account the fundamental importance of reference sites, sampling strategies, equipment and analytical procedures involving gamma spectrometry. The SWMCNL forged new strategic research partnerships with institutions in IAEA Member States (e.g. Nigeria, Slovenia, Switzerland and Yemen) in order to adapt and refine FRN based methodologies for their specific agro-environmental conditions. These improved and tested methodologies were disseminated to Member States through the CRP D1.20.11 on Integrated Isotopic Approaches for an Area-wide Precision Conservation to Control the Impacts of Agricultural Practices on Land Degradation and Soil Erosion and a regional TC project in Latin America entitled Using Environmental Radionuclides as Indicators of Land Degradation in Latin American, Caribbean and Antarctic Ecosystems (ARCAL C).

A methodology was developed and field tested to separate the soil evaporation (E) and crop transpiration (T) components of total evapotranspiration (ET) using cavity ring-down spectroscopy (CRDS). This laser based instrument allows simultaneous and continuous measurements of the concentration and isotopic composition (δ^{2}H, δ^{18}O) of water vapour under field conditions with a precision comparable to isotope-ratio mass spectrometry (IRMS). In addition, a vacuum distillation methodology was developed for extracting water from soil and plant samples for isotope analysis that greatly simplifies and streamlines the previously employed low temperature freezing method. Accurate and reproducible results were obtained for both sand and clay soils (with the precision of CRDS being <0.1‰ for δ^{18}O and <0.5‰ for δ^{2}H), giving >99% recovery of the soil water within a greatly shortened extraction time of 90 minutes. Taken together, the use of CRDS and the improved water extraction methodology provides a greatly enhanced and streamlined analytical capability to investigate water use efficiency that could only be dreamt of a few years ago.

Carbon isotope discrimination (CID, Δ^{13}C) during photosynthesis has been used as a surrogate of the water use efficiency of crops and their tolerance to drought and salinity stress. It was found that CID is greatly affected by the timing of the stress during crop development that can result in variable and unpredictable relationships between yield or yield components and CID. Therefore the possibility that ^{18}O could provide a more robust indicator of the resilience of crops to abiotic stress was investigated. Initial results indicate that ^{18}O is a more consistent indicator of drought stress in wheat than CID. The use of standard IRRI hydroponics to evaluate CID in rice cultivars known to be susceptible and tolerant to salinity was also evaluated against soil based paddy conditions in collaboration with the Joint FAO/IAEA Division's Plant Breeding and Genetics Laboratory (PBGL), Seibersdorf. It was concluded that the atypical root architecture of hydroponic rice and its increased sensitivity to salinity

compared to soil cultured paddy rice makes it unsuitable as a methodology for evaluating CID, except for initial short term screening of large populations of germplasm.

The SWMCNL conducted training activities to support technology transfer under various TC projects. Eighteen fellows, two scientific visitors and one intern were trained in the use of isotopic techniques in agricultural water management, fertilizer use efficiency, biological nitrogen fixation and soil degradation assessment.

Service activities performed by the SWMCNL included 7320 stable isotope and 420 radioisotope measurements to support CRPs, TC projects, training activities and in-house applied R&D. Eleven publications (six journal articles, four conference abstracts and one book chapter) were produced. One IAEA training manual was prepared on the use of fallout radionuclides (FRNs) to assess erosion and sedimentation processes. This manual will provide a much needed hands-on update of significant scientific and technological developments in the area of FRN applications.

STAFF

Name	Title	Email	Extension
Hardarson, Gudni[a]	Laboratory Head	G.Hardarson@iaea.org	28277
Adu-Gyamfi, Joseph	Soil Scientist/Plant Nutritionist	J.Adu-Gyamfi@iaea.org	28263
Mabit, Lionel	Soil Scientist	L.Mabit@iaea.org	28271
Mayr, Leopold	Sr. Laboratory Technician	L.Mayr@iaea.org	28305
Arrillaga, José Luis	Sr. Laboratory Technician	J.L.Arrillaga@iaea.org	28306
Aigner, Martina	Sr. Laboratory Technician (50%)	M.Aigner@iaea.org	28212
Heiling, Maria	Sr. Laboratory Technician (50%)	M.Heiling@iaea.org	28272
Toloza, Arsenio	Laboratory Technician	A.Toloza@iaea.org	28203
Jagoditsch, Norbert	Technical Attendant	N.Jagoditsch@iaea.org	28422
Linic, Sasa	Consultant	S.Linic@iaea.org	28287
Lorenz, Anne	Senior Secretary	A.Lorenz@iaea.org	28274

[a] Retired in December 2010.

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Fallout radionuclide methodologies

To date, most studies involving the use of FRNs (^{137}Cs, $^{210}Pb_{ex}$, ^{7}Be) to estimate soil redistribution have been conducted at the scale of individual fields, although some work has been undertaken in small basins ranging from a few hectares to several square kilometres in area. In 2010, the SWMCNL contributed to one of the major challenges for the further development of the FRNs methodology by up-scaling to the watershed level. One of the possible options favoured and developed by the SWMCNL was to analyse the watershed under investigation using GIS tools and then to subdivide it into subareas or classes (isosectors) representing similar agro-environmental conditions (e.g. soil, slope, land use). Representative agricultural areas can then be selected in each class, and sampled for application of FRN measurements. The results can then be generalized to the different classes and extrapolated to the entire watershed. This approach permits the establishment of a sediment budget, including an assessment of the net sediment output and therefore the sediment delivery ratio (SDR). A clear protocol has been presented in the 2010 FAO/IAEA Soils Newsletter to scale up the use of FRN from the field to the watershed level. However, this approach needs to be developed further and validated under a range of different agro-environmental conditions (climate, soils, topography and cropping systems). Therefore, the SWMCNL established new strategic research partnerships with scientists in institutions in both developed and developing IAEA Member States who were enthusiastic about adapting, developing and applying FRN methodologies tailored to their specific agro-environmental conditions. For example, a new approach to assess background FRN activity in reference sites was validated in Slovenia, and for the first time FRN tracer methodologies were tested in Nigeria, Switzerland and Yemen.

Agricultural water management

Evaporation (E) and plant transpiration (T) are major components of water use in agriculture. Using conventional methods, it is generally only possible to measure E and T collectively (i.e. E + T). However, it is essential to obtain individual measurements of E and T in order to improve water use efficiency in agriculture, because the dual objective is to reduce E and to increase T through improved soil and crop management practices. A stable isotopic method that allows E and T to be determined separately was developed and tested. The method is based on the measurement of the isotopic composition ($^{18}O/^{16}O$) and ($^{2}H/^{1}H$) in the evaporated + transpired water vapour surrounding the plant canopy, in the soil water and in the plant xylem sap.

Estimating E and T under field conditions

Cavity ring-down spectroscopy (CRDS) equipment shared with the Food and Environment Protection Laboratory (FEPL) was used to produce continuous water vapour isotopic measurements in air sampled at different heights above an experimental crop of maize. Preliminary results indicated that T was 81% of ET in the mature crop, indicating high water use efficiency in late summer due to greater canopy cover of the soil and a well established root system. Further developmental work is required to test the methodology in different crops

with respect to growth stage, sampling height and sampling intensity. This methodology is currently being applied in a CRP on Managing Irrigation Water to Enhance Crop Productivity Under Water-Limiting Conditions: a Role for Isotopic Techniques and was further tested during a field campaign carried out in China in 2010.

Methodology to extract water from soil and plants for stable isotope ratio ($^{18}O/^{16}O$ and $^{2}H/^{1}H$) analyses by CRDS

In the past, isotopic measurements of water in soil and plant material could only be achieved by a tedious and complicated process involving trapping by low temperature freezing and subsequent analysis by IRMS. A vacuum distillation system was developed to replace the old methodology, and was tested on sand and clay soils over a range of moisture levels from field capacity to permanent wilting point. Accurate and reproducible results were obtained for both sand and clay soils (with precision of the CRDS being <0.1‰ for $\delta^{18}O$ and <0.5‰ for $\delta^{2}H$), giving >99% recovery of the soil water within 90 minutes (Fig. 7).

FIG. 7. The vacuum distillation system for extracting water from soil and plant samples for oxygen isotope and deuterium measurements.

Crop tolerance to abiotic stress (drought and salinity)

Oxygen ($\delta^{18}O$) and carbon ($\Delta^{13}C$) relationships in wheat

Plants differ in their ability to discriminate against the heavier carbon isotope, ^{13}C, in favour of the lighter ^{12}C during photosynthesis. This carbon isotope discrimination (CID) is related to the water use efficiency of the plant and can therefore be used as a surrogate marker for the identification of drought or salinity tolerant varieties. The problem with CID is that relationships with crop yields or components of crop yield can vary (positive or negative) depending on the timing and severity of the stress during crop development, the part of the plant that is sampled as well as the crop itself. Therefore a more robust technique is required that is less sensitive to such variations.

Plant $^{18}O/^{16}O$ ratios have been shown to vary with the transpiration rate, which is closely related to carbohydrate assimilation during photosynthesis, and could therefore have potential use as an indicator of crop tolerance to drought. Therefore, the relationship between the oxygen ($^{18}O/^{16}O$ ratios, expressed as $\delta^{18}O$) and carbon ($^{13}C/^{12}C$ ratios, expressed as $\Delta^{13}C$) isotopic composition in wheat was investigated. Wheat plants were grown in the field and were subjected to different water stress levels until maturity. Dry leaf and grain samples were analysed for $\Delta^{13}C$ and $\delta^{18}O$ using IRMS, and $\Delta^{13}C$ values were converted to CID ($\Delta^{13}C$) values. Preliminary results showed that variations in $\delta^{18}O$ in grains could be a more robust indicator of the tolerance of crops to drought compared with the currently used CID. More research is required to compare the two isotopic techniques in evaluating crop plants for their tolerance to drought.

Salinity tolerance and carbon isotope discrimination ($\Delta^{13}C$) in rice (collaboration with the PBGL)

Standard methodologies for evaluating the tolerance of rice genotypes to salinity were developed in the glasshouse under hydroponic conditions at the International Rice Research Institute (IRRI). Since it is known that plant root development in hydroponics is radically different from that in soil culture, it was necessary to re-evaluate the CID methodology under soil based paddy conditions. Four rice varieties, Pokkali, salt and drought tolerant, Bicol, moderately tolerant, IR29, salt susceptible (from IRRI), and STDV, a moderately tolerant mutant developed by the PBGL, were subjected to three salinity treatments (0, 6, and 12 dS/m) using NaCl. Plants in both hydroponics and simulated paddy conditions were harvested and analysed for the $^{13}C/^{12}C$ ratios by IRMS. Plants grown under simulated paddy culture could survive until maturity at 12 dS m^{-1} while the solution culture plants at 12 dS m^{-1} died within 32 days after treatment, presumably due to the absence of the soil buffering capacity. This result illustrates that while IRRI standard hydroponics may be useful as an initial screening tool for large germplasm populations, it cannot substitute for soil based paddy conditions in a final evaluation of CID.

CAPACITY BUILDING

Fellowship training

Group training on managing soil and water in salt affected soils for enhanced crop productivity using isotopic techniques was organized for five fellows from Iraq from 30 August to 8 October 2010 at the SWMCNL. The programme consisted of: (i) lectures on soil and water salinity and how crop growth is affected; (ii) practical glasshouse and field training on the monitoring of soil and water salinity and soil–plant–water interactions (Fig. 8); and (iii) data analysis and interpretation. The CID technique was used to assess the tolerance of three crops (paprika, rice and sorghum) to soil and water salinity.

Similarly, three fellows from Iraq and one from Jamaica received five weeks of training in the assessment of soil degradation. The objective was to give the fellows a sound working knowledge of the use of FRNs with an emphasis on the application of ^{137}Cs to study soil erosion. This training aimed to transfer knowledge on tracking and quantifying soil redistribution at various spatial and temporal scales (from field to watershed) to evaluate the effectiveness of soil conservation measures. The training included lectures and practical exercises on basic concepts and training on the use of FRNs as well as field work and sample collection in Seibersdorf and in the Mistelbach watershed. The training also included soil sample preparation, gamma spectrometry, the use of FRN conversion models (focus on ^{137}Cs), data treatment and mapping

Other training activities (collaboration with FEPL)

The SWMCNL supported the FEPL in conducting a practical training course on how land management practices (e.g. conservation agriculture) influence pesticide behaviour and how these practices can help reduce the impact of pesticides on the environment.

The principles of spatial variability in soil properties and how this variability can be measured and statistically analysed were explained. A total of 10 participants from Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador and Uruguay attended the training course.

During the third RCM of CRP D5.20.35, coordinated by the Food and Environmental Protection Section, the SWMCNL gave an introductory lecture on the combined application of radionuclide and stable isotopic techniques to estimate soil erosion at the watershed scale, and modelling transport processes affecting pesticide movement across the landscape.

The SWMCL collaborated with the FEPL by training staff on the extraction of water from soil and plant samples for stable isotope ratio ($^{18}O/^{16}O$ and $^{2}H/^{1}H$) analyses that could be easily modified for food samples, and also assisted in the oxygen stable isotope analysis using CRDS. This technology transfer will benefit Member States via the CRP on food traceability using isotopic fingerprints.

FIG. 8. Training of fellows on the use of cavity ring-down spectroscopy (CRDS) equipment at Seibersdorf.

Training manual

The SWMCNL responded to the increasing attention being paid by Member States to land degradation worldwide, by producing a training manual based on FRN measurements that can be used by FAO and IAEA Member States as a practical guide for (i) tracing and assessing soil degradation processes associated with soil erosion at different temporal scales, and (ii) evaluating the effectiveness of soil conservation strategies to ensure sustainable land management and productivity in agricultural systems. The manual also includes guidance on methods and instrumentation that are important for quantifying FRNs. The manual — to be submitted in 2011 to the IAEA Publications Committee — consists of nine sections with contributions from 20 authors from eight Member States (Austria, Canada, Chile, Hungary, Morocco, Slovakia, Switzerland and the United Kingdom) and from IAEA staff. This training manual will provide clear technical guidance in the application of FRNs to an audience of scientists and technicians from different disciplines (e.g. soil science, ecology, agronomy, engineering) as well as extension workers, undergraduate and graduate students, and staff of non-governmental organizations and other stakeholders involved in sustainable agricultural development at local, national, regional and international levels.

Analytical and quality assurance services

The SWNCNL provided isotope analyses to CRPs and TC projects in Member States where analytical facilities are not currently available, to training activities as well as to in-house applied R&D. During 2010, 420 soil and plant samples were analysed for ^{137}Cs, ^{40}K, ^{226} Ra and ^{232}Th, 2539 for ^{15}N-enriched, 1111 for ^{15}N natural abundance, 2946 for ^{13}C and 698 for ^{18}O. In addition the laboratory supplied ^{15}N-enriched plant reference materials and assisted in delivering quality assurance services to 15 participants; it also assisted nine participants through proficiency testing of ^{13}C natural abundance samples in collaboration with the Wageningen Evaluating Programs for Analytical Services (WEPAL). Eleven and seven laboratories returned acceptable results for the ^{15}N-enriched and ^{13}C-natural abundance samples, respectively.

PUBLICATIONS

Jankong, P., Mabit, L., Toloza, A. and Zupanc, V. (2010). Anthropogenic and geogenic radionuclides content in an undisturbed Slovenian forest soil (abstract). In: Geophysical Research Abstracts, Volume 12, European Geosciences Union — General Assembly 2010. EGU2010-3124.pdf.

Junge, B., Mabit, L., Dercon, G., Walling, D.E, Abaidoo, R., Chikoye, D. and Stahr, K. (2010). First use of the ^{137}Cs technique in Nigeria for estimating medium-term soil redistribution rates on cultivated farmland. Soil and Tillage Research, 110(2): 211–220.

Mabit, L. (2010). Measurement of soil water erosion in Africa: the potential support provided by nuclear techniques (abstract). In: Geophysical Research Abstracts, Volume 12, European Geosciences Union — General Assembly 2010. EGU2010-3098.pdf.

Mabit, L., Benmansour, M. and Nouira, A. (2010). A case study of soil erosion and sedimentation magnitudes in Morocco using ^{137}Cs & ^{210}Pbex (abstract). In: Geophysical Research Abstracts, Volume 12, European Geosciences Union — General Assembly 2010. EGU2010-3105.pdf.

Mabit, L. and Bernard, C. (2010). Spatial distribution and content of soil organic matter in an agricultural field in Eastern Canada, as estimated from geostatistical tools. Earth Surface Processes and Landforms, 35: 278–283.

Mabit, L., Klik, A. and Toloza, A. (2010). Radioisotopic measurements (^{137}Cs and ^{210}Pb) to assess erosion and sedimentation processes: Case study in Austria. In: Zdruli, P., Pagliai, M., Kapur, S. and Faz Cano, A. (Eds), Land Degradation and Desertification: Assessment, Mitigation and Remediation, Springer, pp. 401–412.

Mabit, L., Martin, P.C., Jankong, P., Toloza, A., Padilla-Alvarez, R. and Zupanc, V. (2010). Establishment of control site baseline data for erosion studies using radionuclides: A case study in East Slovenia. Journal of Environmental Radioactivity, 101(10): 854–863.

Rabesiranana, N., Rasolonirina, M., Solonjara, A.F, Raoelina, A. and Mabit, L. (2010). Preliminary study of a radiological survey in an abandoned uranium mining area in Madagascar (abstract). In: Geophysical Research Abstracts, Volume 12, European Geosciences Union — General Assembly 2010. EGU2010-3155.pdf.

Shirazi, M.U., Adu-Gyamfi, J.J., Ram, T., Bachiri, H., Rasyid, B., Rehman, A., Khan, M.A., Mujtaba, S.M., Ali, M., Shreen, A. and Mumtaz, S. (2010). Selection of some suitable drought tolerant wheat genotypes using carbon isotope discrimination (CID) technique. Pakistan J. Botany, 42(5): 3639–3644.

Suwa, R., Hakata, H., Hara, H., El-Shemy, H.A., Adu-Gyamfi, J.J., Nguyen, N.T., Kanai, S. and Fujita, K. (2010). High temperature effects on photosynthate partitioning and sugar metabolism during ear expansion in maize (*Zea mays* L.) genotypes. Plant Physiology and Biochemistry, 48: 124–130.

Zupanc, V. and Mabit, L. (2010). Nuclear techniques support to assess erosion and sedimentation processes: preliminary results of the use of ^{137}Cs as soil tracer in Slovenia. Dela, 33: 21–36.

11-48791