Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

FAO/IAEA
Agriculture & Biotechnology Laboratories

Activities Report **2012**

IAEA Laboratories Seibersdorf, International Atomic Energy Agency, Vienna, Austria

CONTENTS

THE ANIMAL PRODUCTION AND HEALTH LABORATORY

EXECUTIVE SUMMARY

The livestock sector contributes about 40% of global agricultural gross domestic product (GDP) and provides, at least in part, a livelihood for about one billion people[1]. Over the last decades, it has developed in a highly dynamic context, characterized by the rapid growth in the demand for livestock products – the 'livestock revolution' – a trend which is driven by rising incomes, urbanization and preferences by the growing middle classes for a diet that includes milk, meat, eggs and other highly nutritious foods. Meeting this trend by improving livestock productivity in developing countries provides unique opportunities for using livestock as instruments for poverty alleviation and for promoting food/nutritional security. Most of the small farmers in the tropics, who represent around 20% of the world's farming population, are relying on livestock activities for their livelihood. Those farmers can benefit from the increase in demand for livestock products only if sufficient emphasis is given to empowering and developing their production capacities through appropriate means, including the control of major animal diseases and improving of the genetic potential of local breeds. With this objective in mind, the Animal Production and Health Subprogramme supports the IAEA and FAO Member States, in particular in the developing world, to increase livestock productivity in a sustainable manner through the development and transfer of technologies for the control of major animal diseases; the improvement of animal genetics resources and animal nutrition; and the preservation of indigenous livestock breeds. Within this context, the Animal Production and Health Laboratory (APHL) conducts research and development (R&D) of tools used in animal breeding strategies and animal disease control, provides support in building capacities for the use of these tools and offers services when requested.

In 2012, APHL continued the R&D activities on capripox to develop new, improved and cheaper molecular-based techniques for the identification of viruses causing this disease. It has developed three methods based on nucleic acid amplification using labelled and unlabelled probes and has started to disseminate these tools.

For peste des petits ruminants (PPR), APHL has provided diagnostic services to nine Member States. Partial gene sequencing was performed on identified viruses and the resulting data were exploited to expand the knowledge on molecular epidemiology of the PPR virus (PPRV). Based on the results that were obtained at APHL, Angola declared its first case of PPR to the World Organisation for Animal Health (OIE).

In 2011, APHL embarked on the development of an irradiated vaccine for the control of trypanosomosis and early irradiation experiments carried out using an X-ray source identified a dose of 250 Gy to be effective in obtaining non-replicating but metabolically active parasites. In 2012, this study was repeated using a cobalt source. It was found that the results achieved with both sources gave comparable results and can be used interchangeably.

[1] World Bank, 2012: Identifying investment opportunities for ruminant livestock feeding in developing countries. November 2012; 181 p

In the animal genetics area, APHL conducted research on the discovery of SNP (single nucleotide polymorphism) markers and the development of genotyping tools to allow the association of genetic variations in immune related genes with disease resistance characteristics in indigenous sheep, goats and chicken: 41 novel SNP markers associated with helminth resistance in sheep and 11 novel DNA markers for genotyping viral resistance/susceptibility in 18 chicken breeds were developed. Initiatives were made to genotype radiation hybrid (RH) panels developed for the goat genome with candidate genes and microsatellite markers so that the first generation whole genome RH map of goat can be constructed and published. APHL also supported Member States in the genetic characterization of indigenous livestock breeds using FAO recommended microsatellite markers as part of the implementation of FAO's Global Plan of Action on Animal Genetic Resources (AnGR). In addition, the global genetic DNA repository of indigenous livestock breeds has been significantly strengthened with more than 900 new DNA samples, a valuable reference material for collaborative animal genetic research.

In addition to the above R&D activities that were continuations of studies initiated in previous years, the APHL initiated work in 2012 on Orf, a parapoxvirus disease of sheep and goats that is zoonotic (i.e. can affect humans), and African swine fever (ASF), a highly contagious swine disease for which no vaccine is yet available. Initial results on Ethiopian samples showed that at least three strains of Orf virus are circulating in this country, while preliminary results on ASF have shown for the first time that virus strains of the genotype I present in Central Africa can be subdivided into sub-genotypes. Such data must be taken into consideration in understanding virus variations and successive outbreaks.

In the domain of capacity building, APHL conducted three training courses in 2012 and organized two ring tests: one on PPR and one on RVF. Furthermore, and in collaboration with the Animal Health Service (FAO, Rome) and the Swiss Institute of Bioinformatics, Switzerland, an e-learning module on gene sequence analysis and molecular epidemiology was developed.

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MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Animal health

Capripoxvirus genotyping tool development

The *Capripoxvirus* (CaPV) genus within the *Poxviridae* family comprises sheeppox virus (SPPV), goatpox virus (GTPV) and lumpy skin disease virus (LSDV), which are responsible for causing capripox disease in primarily sheep, goat and cattle, respectively. The epidemiology of the disease is complex and the three viruses involved in the diseases are not strictly host specific. This creates an urgent need for routine differentiation tools to allow for accurate identification of the pathogen in order to define and implement better control strategies. APHL has developed three methods based on nucleic acid amplification using labelled and unlabelled probes and has started to disseminate these tools to specialist laboratories. However, a more extensive usage will require a more cost effective method that is affordable also to most Member State veterinary laboratories. With this in mind, a research project on the development of molecular tools for the genotyping of CaPVs using nucleic acid amplification with unlabelled probes was initiated in 2011. This method was further evaluated in 2012 using more than 60 clinical specimens from capripox suspected animals collected from different geographical locations, including Africa and Asia. The method was able to successfully genotype and to assign the pathogens from these specimens to one of the three CaPV groups: SPPV, GTPV and LSDV. The robustness of the method was evaluated by analysing the data with a special method to study nucleic acid melting characteristics: the high resolution melting curve analysis (Fig. 1).

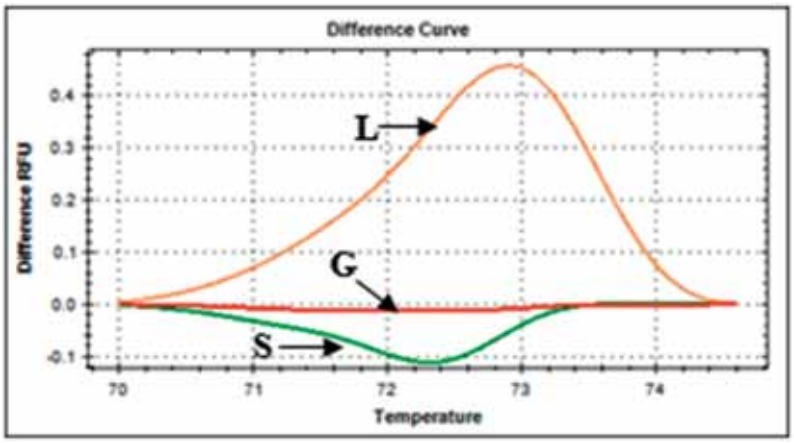

FIG. 1. High-Resolution melting curve analysis of the three genotypes of Capripox viruses (G=GTPV, S=SPPV and L=LSDV) using the Precision Melt Analysis™ software

Molecular characterization of Orf virus

With a view to developing a multiple pathogen detection method for ruminant poxviruses, the APHL has begun generating genetic information on other poxviruses that affect ruminants. Outbreak samples collected from Ethiopian goats and sheep were submitted for molecular characterization of the Orf virus, a member of the Parapoxvirus genus. The targeted gene was subjected to PCR amplification and sequencing. A phylogenetic tree was constructed to compare these data with those of other Orf virus sequences retrieved from GenBank®. Preliminary finding are presented in Fig. 2. The results show that at least three different strains of Orf virus are circulating in Ethiopia.

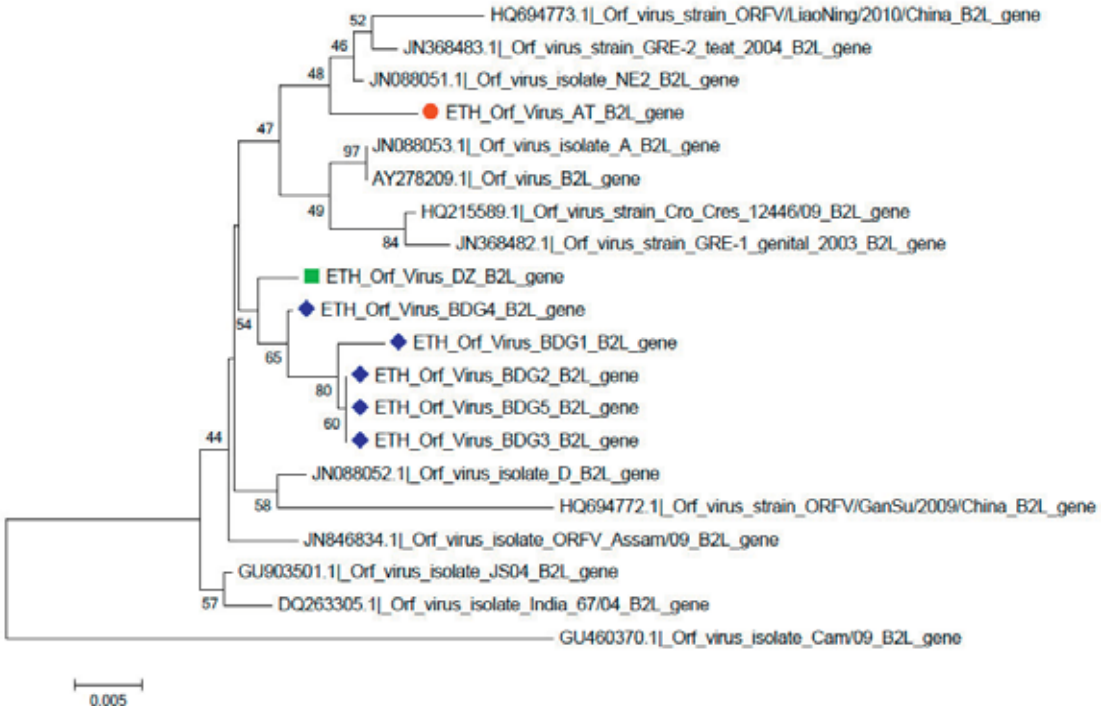

FIG. 2. Phylogenetic analysis of Orf viruses based on the nucleotide sequences of the major envelope protein (B2L gene).

Development of Pan-pox virus detection method

The APHL has recently started a research program on multiple pathogen detection methods that can be applied to specimens according to the clinical signs. Multiple pathogen detection is a valuable approach to diagnose diseases using an assay that can target several pathogens in the same sample. With this approach, pathogens are selected based on the similarity of the symptoms they produce in animals or humans. By running such an assay, which will contain a specific detection system for each of the pathogens of the assay panel, it will be possible to identify the pathogen(s) responsible for the symptom. With that objective in mind, APHL set out to develop a pan-pox virus detection method for the specific identification, in a single tube, of different poxviruses of veterinary and public-health importance. This assay is currently under evaluation to determine assay performance and specificity.

Molecular epidemiology of African swine fever virus isolates from Central and West Africa

African swine fever (ASF) is a fatal haemorrhagic and infectious disease of pigs caused by a DNA virus. It persistently infects its natural hosts, warthogs, bush pigs and soft ticks of the Ornithodorous genus, with no disease signs. ASF is considered endemic in most Sub-Saharan Africa but with an increasing number of outbreaks not only in African countries. Some contributing factors to this exponential spread are the infection cycle, the lack of vaccine or drugs to prevent or treat ASF infections. Today, all 22 genotypes of ASF are found in Africa. This situation calls for a thorough molecular epidemiology study to understand the spread and, more interestingly, to formulate a candidate vaccine derived from currently existing isolates.

With this objective, the APHL initiated a study to characterize ASFV strains collected from different outbreaks that occurred in 2011 and 2012 in Central Africa and from 1983 to 2008 in Western Africa. This study will help to better understand the movement and evolution of this virus in these regions and provide the basis to develop a strong regional programme for the control of ASF. Five ASFV genes were amplified and sequenced: the B646L gene (encoding the vp72 protein), the B602L gene in the central variable region of the viral genome (encoding the vp9RL protein), the CP204L (encoding the vp30 protein), the E183L gene (encoding the vp54 protein) and the E184L gene (encoding the vpE184L protein). ASF samples from Cameroon, Central African Republic, Chad, Democratic Republic of Congo (DRC) and Senegal were analysed.

The sequencing data generated were used for phylogenetic analysis together with the sequences of other ASFV strains retrieved from public databases. An example of the sequence comparison of the Cameroon and Chad samples using partial VP72 gene sequences is shown in Fig. 3. The results indicate that all samples collected from Cameroon and Chad are clustering in the ASFV VP72 genotype I. Similar results were found with samples collected in different years and from different outbreaks in Senegal, which are also clustering in the genotype I. These results were further confirmed by a phylogenetic analysis using the P54 protein gene sequence. Interestingly, with the sequence information of the central variable region of the virus genome, it was possible to further classify Cameroon ASFV isolates into subtypes within the genotype I. This analysis revealed that three different viral strains were involved in the recent ASF outbreaks in Cameroon. Viruses identified in two samples collected from Chad are similar to two of the Cameroon genotype I subtypes.

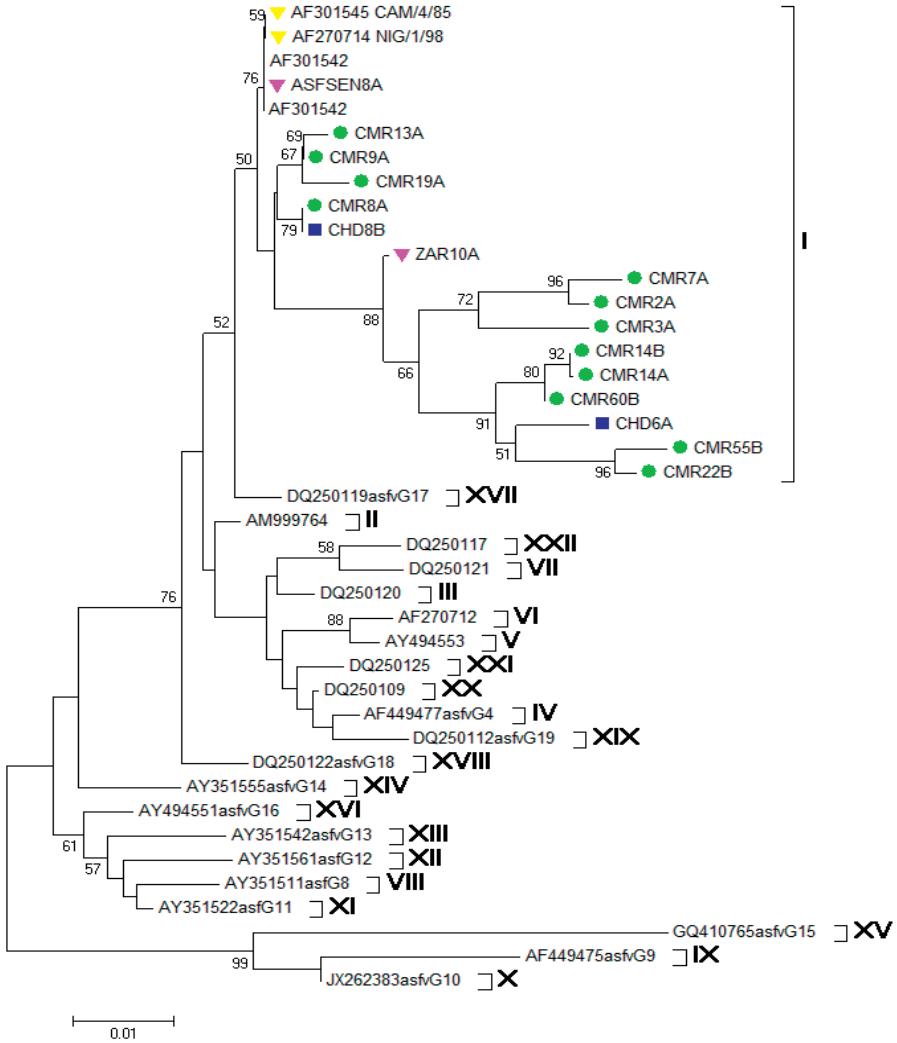

FIG. 3. Phylogenetic tree constructed using partial p72 gene sequence data from Cameroon and Chad isolates and sequences available in Genbank®.

A sample collected from DRC in 1989 was also found to be of the VP72 genotype I. However, it had a different profile when compared to those involved in the recent outbreaks in Cameroon and Chad. Although this work is in progress, the preliminary findings show that multiple introductions of ASF involving several ASFV strains do occur in these countries. It would therefore be of great importance to understand the mechanism of the genetic evolution of this virus. In 2013, the ASF work will continue with the investigation of new outbreaks covering more geographical locations and the generation of more genetic information on ASFV. This will be done in close collaboration with counterparts in Member States. It is expected that this information will help develop more effective control strategies.

Molecular epidemiology of PPR

Following the development in 2009 of a new cell line that is very sensitive to PPRV, the APHL regularly receives field isolates from Member States requesting confirmation of suspected PPRV outbreaks. In 2012 APHL received 412 samples from nine countries (see table 1). In the case of DRC, the samples were collected in different years: from 2009 to 2012.

The request was to identify possible temporal and spatial changes to PPRV circulating in the country during this 4 year period. From the sequence data of two different PPRV genes an analysis clearly confirmed that all isolates were of the same genotype, genotype IV (NB: to date all PPRV strains are classified into 4 genotypes: I, II, III and IV). While only genotype IV is found in Asia, all 4 genotypes are prevalent in Africa. PPRV identified from Ethiopian samples, received and analysed at APHL in 2012, were also of genotype IV. Identified in Sudan, in Eritrea and now in Ethiopia, it seems that this PPRV genotype is progressing throughout the Horn of Africa, a region that was known, until 2008, to be infected only by viruses of genotype III. In fact, genotype IV viruses are also extending into Central Africa. In addition to the DRC, the PPRV identified in samples received from Angola were also genotype IV. Based on the results that were obtained at APHL, Angola declared its first case of PPR to the OIE.

TABLE 1. FIELD ISOLATES FROM MEMBER STATES REQUESTING CONFIRMATION OF SUSPECTED PPRV OUTBREAKS

Country of origin	Number of samples received
Angola	35
Benin	23
Democratic Republic of Congo	37
Ethiopia	27
Gambia	14
Mali	47
Sierra Leone	60
Sudan	159
Turkey	10
TOTAL	**412**

Development of an irradiated vaccine for the control of trypanosomosis

In 2011, APHL embarked on the development of an irradiated vaccine for the control of trypanosomosis. Irradiation experiments with an X-ray dose of 250 Gy was effective in obtaining non-replicating but metabolically active parasites. In 2012, this study was repeated using a cobalt source and showed that a dose of 250 Gy using the cobalt source produced results comparable to those of the X-ray source and that both sources of irradiation can be used interchangeably. In both studies, the irradiated parasites were submitted to microscopic observation and the analysis of messenger RNA (mRNA) using quantitative PCR. In order also to characterize metabolically active but non-dividing parasites a proliferation assay, using a fluorescent dye that measures the depletion of fluorescence as parasites divide, was performed using flow cytometry. Results have so far shown that a *Trypanosoma evansi* culture of 3×10^5 stained with 5 µM of CFSE, a fluorescent cell staining dye, can be used to determine the proliferation of parasites over a 5-day period. We are now in the process of assessing the protease activity of irradiated parasites, protease activity being the main pathogenesis factor

of trypanosomosis. Parasites that have been irradiated with 250 Gy from an X-ray source and have been determined to be metabolically active using microscopy, quantitative PCR, flow cytometry and protease activity will then be used for immunisation in mice before evaluating protection when vaccinated mice are challenged with infective parasites.

Animal genetics

Genetic variation in the control of resistance to infectious diseases in small ruminants for improving animal productivity

Gastro-intestinal parasitic infestations incur huge economic losses to poor and marginal farmers rearing sheep and goat across the world. Breeding programmes with the goal of enhancing host resistance to parasites will help alleviate this problem in the long term. Exploration of genetic variation within the whole genome and in specific candidate genes involved in innate and adaptive immune pathways may help identify DNA markers associated with parasite resistance characteristics. Such DNA markers may help to develop marker assisted selection programmes for the breeding of indigenous sheep and goat breeds with enhanced host resistance to such parasites.

Genetic polymorphisms of candidate genes involved in immune pathways

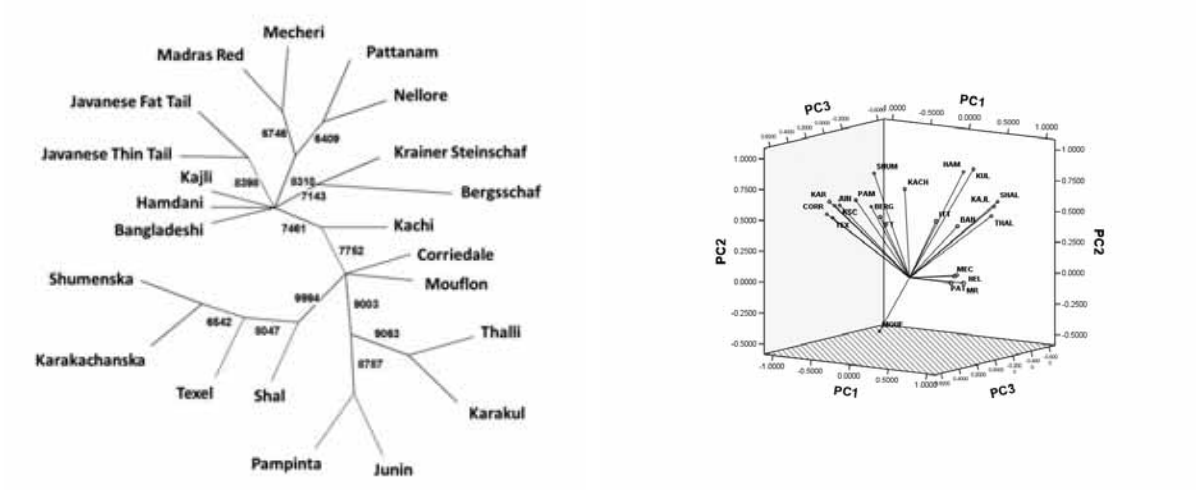

FIG. 4. Phylogenetic tree based on pair-wise Nei's Standard Genetic Distance at 41 SNP loci (left), and principal components analysis of pair-wise F_{ST} (F statistics-genetic differentiation) at 27 SNP loci (right)

A total of 60 SNPs (single nucleotide polymorphisms) markers were identified from 49 candidate genes involved in various immune pathways, of which 58 SNPs qualified for the development of genotyping assays. In order to delineate the underlying genetic diversity of different indigenous sheep breeds with respect to these candidate genes, 713 animals belonging to 22 indigenous sheep breeds were genotyped at 41 SNP loci. Analysis of genotypic data from all 22 sheep breeds was performed to establish the immune gene diversity based genetic relationship of sheep across Asia, Europe and Latin America. It indicated that sheep breeds were clustering on the lines of broader geographical location of their native habitat (Fig. 4). A preliminary association analysis of genotypes in about 100 animals revealed significant

association of certain SNP markers with parasite resistance characteristics, like faecal egg count, packed cell volume and body weight.

Genetic variation of pattern recognition receptor genes involved in innate immunity

Pattern recognition receptors (PRR) are surface receptors involved in the host innate immune system and recognize microbe specific molecules called pathogen associated molecular patterns (PAMPs). It has been hypothesized that differences in these receptors confer on host animals the ability to resist different pathogens, including parasites. Genetic variations within many PRR genes have been found to be significantly associated with genetic disease resistance in various livestock species. However, information on genetic polymorphisms within many of these PRR genes is limited in sheep and goats. Identification of novel SNPs within the candidate genes of different PRRs will not only be helpful for establishing their underlying genetic variations but also for identifying DNA markers for disease resistance. At APHL, more than 100 sets of primer pairs were designed to sequence and screen different PRR genes to detect novel SNPs in a panel of eight unrelated sheep. More than 200 novel SNP markers were discovered in 2012 and SNP discovery in other PRR genes will be continued in 2013 for association studies on parasite resistance in sheep and goat.

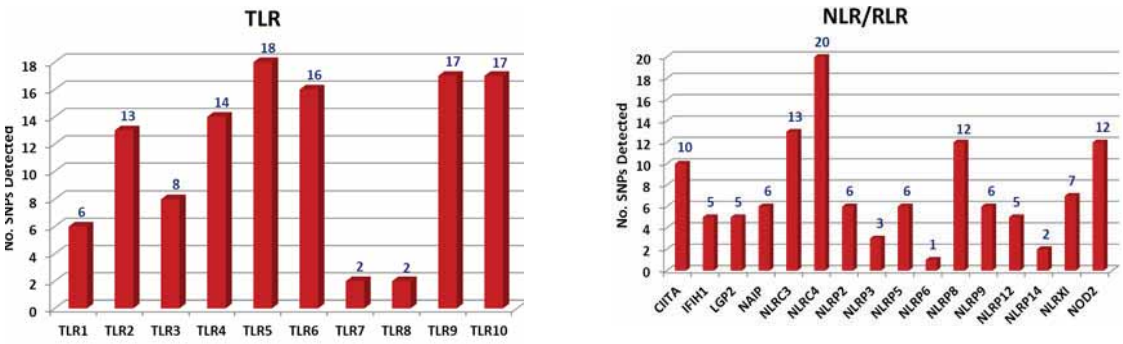

FIG. 5. *Number of SNPs detected in different pattern recognition receptor genes; Toll-like receptor (TLR; left) and Nod/Rig I Like receptor (NLR/RLR; right).*

Radiation hybrid panel mapping of goat genome

Although goat is an important livestock, genomic information available on them is relatively poor as compared to other major species such cattle and sheep. For example, the resolution of the goat gene map currently available is very low with only few markers being mapped to each of the 30 pairs of chromosomes. APHL, in collaboration with other institutions, initiated in 2009-2010 the development of radiation hybrid panels for gene mapping in goats. A 5000 rad goat–hamster whole-genome radiation hybrid panel was generated and preliminarily characterized. In 2012, APHL initiated the process of mapping candidate genes related to gastro-intestinal resistance and those involved in various immune pathways, including pattern recognition receptor genes. About 140 gene markers, including 92 candidate genes involved in various immune pathways, were targeted for genotyping. Of these markers, optimization of genotyping protocols for more than 100 has been completed. About 250 microsatellite markers have been identified for use in the mapping of the goat RH panels. Further genotyping of other microsatellite markers is in progress.

Genetic characterization of indigenous chicken breeds in search of unique variation in immune related genes

Indigenous chicken populations around the world possess wide genetic diversity, and searching for beneficial mutations across important immune related genes will be helpful for improving bird resistance to diseases. The myxovirus resistance gene (Mx/resistance) is one of the important candidate genes with respect to genetic resistance to avian influenza. The amino acid variation of Asn (allele A) at position 631 in the sequence of this protein has been found to be specific to positive antiviral Mx/resistance, while that of Ser (allele G) is specific to negative Mx/susceptible. A total of 610 birds from 18 chicken populations, including 13 indigenous breeds (seven from Europe and six from Asia) and five commercial strains, were screened for nucleotide variations at the S631N locus. Interestingly, all the chicken breeds/strains developed for egg production were found to have a high frequency of the resistant allele (allele A), while those developed for meat were found to have a high frequency of the susceptible allele (allele G) irrespective of geographic location and of being indigenous or commercial birds. Apart from the S631N locus, 11 other SNP loci discovered in different regions of the Mx gene were genotyped to assess the genetic variation in different chicken breeds. Genotypic data analysis showed two major clusters revealing differences in the genetic structure of indigenous chicken from Asia and Europe with respect to this important candidate gene for disease resistance.

Supporting Member States to implement FAO's Global Plan of Action on Animal Genetic Resources

Domestic animal genetic resources (AnGR) represent a unique source in responding to present and future needs in livestock production. Responding to the rapid erosion of these resources globally, and in developing countries in particular, the international community agreed to adopt the FAO's Global Plan of Action (GPA) on AnGR. The IAEA, through its national and regional technical cooperation (TC) projects, support Member States in implementing the GPA, especially in terms of capacity building and technical support for genetic characterization of livestock using molecular DNA technologies. APHL provides technical support in microsatellite genotyping of native breeds, data analysis and interpretation of results. It is currently supporting the Arab Asian Member States in molecular genetic characterization of small ruminant genetic resources under a regional TC project on "Improving the reproductive and productive performance of local small ruminants by implementing reliable artificial insemination programmes".

Global reference genetic repository of livestock breeds for animal genetic research

For its global reference genetic repository, APHL collects, preserves and maintains genomic DNA from distinct breeds of various livestock species, including cattle, sheep, goat, chicken, alpacas, rabbits, etc. The main objective of establishing the genetic repository is to encourage and strengthen collaborative animal genetic research across different countries and regions. The genetic repository is constantly strengthened by the addition of new DNA samples. More than 900 samples from 35 different populations of seven livestock species were added to the repository during 2012.

CAPACITY BUILDING

E-learning

The vast diversity of the pathogens affecting livestock demands very specific diagnostic procedures in the identification and characterization of each pathogen. In this context, an enormous amount of sequence and genotype data is being generated on pathogens that is useful in understanding their pathogenicity and molecular epidemiology. Use of such data in developing efficient molecular diagnostic tools needs a basic understanding of phylogenetic analysis that will help improve the management of animal diseases. The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, together with the Animal Health Service (FAO, Rome) and the Swiss Institute of Bioinformatics, Switzerland, has therefore launched an e-learning module on "The phylogenetics of animal pathogens: basic principles and applications" *(http://viralzone.expasy.org/e_learning/partners.html)***.**

This e-learning tool contains an introduction to phylogenetics for the better management of animal diseases, and lessons on tools, the building and interpreting of trees, and their application to veterinary diagnostics. The module aims to prepare laboratory technicians, veterinarians and molecular epidemiologists from diagnostic and research laboratories in Member States to be self-sufficient in data analysis and interpretation.

Training courses

Regional training course on "Early and rapid nuclear and nuclear-related diagnostic and tracing technologies for African and classical swine fever"

The training course took place in the IAEA's laboratories at Seibersdorf, Austria from 21-25 May 2012. This training was supported by the IAEA TC Department under the regional project RER/5/016 on "Supporting coordinated control of transboundary animal diseases with socioeconomic impact and that affect human health". The course agenda included theoretical lectures, practical sessions and epidemiological simulation examples given by renowned experts in the field of diagnostics and epidemiology of African and classical swine fever. The laboratory practical sessions focused on established and recognized diagnostic procedures, harmonized for sensitive and rapid diagnosis of the two diseases, and useful within national and regional surveillance and control plans. Lectures and practical classes were delivered by trainers from the Universidad Complutense de Madrid (Spain) with the technical assistance of the APHL. Sixteen participants, specialists in veterinary diagnostics from Albania, Azerbaijan, Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Kyrgyzstan, Montenegro, Tajikistan, The Former Yugoslav Republic of Macedonia and Turkey, with Austria as observers, attended this course.

Regional training course on "Major Transboundary and Zoonotic Animal Diseases: Early detection, Surveillance and Epidemiology"

The course was held at the Laboratoire National Vétérinaire (LANAVET), Garoua (Cameroon) from 23 July to 3 August 2012**.** The course was jointly supported by the USAID funded "Identify project" and the South Africa funded "African renaissance fund (ARF)". This training course focused on African swine fever, rabies and capripox. It consisted of practical sessions and theoretical lectures on the diagnosis, epidemiology and control strategies of

these diseases. The practical session for capripox diagnosis focused on molecular methods developed at APHL for capripox virus detection and differentiation. Lectures and practical sessions were delivered by trainers from the Universidad Complutense de Madrid (Spain) for ASF, the Istituto Zooprofilattico Sperimentale delle Venezie (Italy) for rabies and the IAEA (Vienna) for capripox. Twenty-one participants from Burkina Faso, Cameroon, Central African Republic, Congo, Equatorial Guinea, Ethiopia, Gabon, Kenya, Mali, Mozambique, Rwanda, Senegal, Tanzania, and Uganda attended the course.

Training course on sequencing and molecular epidemiology of animal pathogens

The training was organized by the IAEA and the FAO through the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, the FAO Animal Health Service and the sub-regional units of the FAO Emergency Centre for Transboundary Animal Diseases (ECTAD) for East Africa and for West and Central Africa. The course, jointly funded by the USAID funded "Identify project" and the South Africa funded "African renaissance fund (ARF)", took place at the IAEA's laboratories in Seibersdorf, Austria on 10-21 December 2012. The following transboundary animal diseases were covered: foot and mouth disease (FMD), avian influenza (AI), Newcastle disease (ND), peste des petits ruminants (PPR), contagious bovine pleuropneumonia (CBPP) and capripox.

The training program contained theoretical and practical sessions on sequencing and molecular epidemiology of animal pathogens causing transboundary animal diseases. In the first week, training focussed on gene amplification using harmonized protocols for selected gene targets, purification of PCR products, samples sequencing and sequencing data handling and management with focus on PPR, capripox and CBPP. The second week emphasized database usage, sequence retrieval and sharing, sequence analysis and phylogeny. This included sequence submission to databases and multiple sequence alignment. Different phylogenetic software were used in sequence analysis and molecular epidemiological studies, and focus was on FMD, AI, NDV, PPR, capripox and CBPP. Lectures and practical sessions were delivered by experts from IZSVe (Instituto Zooprofilattico Sperimentale delle Venezie, Italy), CODA-CERVA-VAR (Veterinary and Agrochemical Research Centre, Belgium), CIRAD (Agricultural Research for Development, France), SIB (Swiss Institute of Bioinformatics, Switzerland) and APHL staff. Twenty participants from ten African countries (Botswana, Cameroon, Democratic Republic of the Congo, Ethiopia, Kenya, Mali, Nigeria, Senegal, Tanzania and Uganda) attended the training course.

Proficiency testing

Ring tests or inter-laboratory proficiency trials are exercises to check, evaluate and "quality control" the performance of participating laboratories in the routine application of a disease diagnostic test. Such ring tests help participating laboratories confirm the correctness of their routine test results and/or improve where and when necessary.

Two years ago, APHL started the organization of ring tests in the field of animal disease diagnosis. In 2012, this exercise focused on (i) the evaluation of the nucleic acid amplification based PPR diagnostics and (ii) the serological diagnosis of Rift Valley fever.

Ring Test 2012: PPR nucleic acid amplification based diagnosis.

In support to the PPR CRP D32026, a ring test was organized to evaluate the nucleic acid amplification-based PPR diagnosis in the counterpart laboratories. Twenty participants (counterparts of the PPR CRP D32026 and the US-AID-funded project "Identify" as well as the High Security Laboratory of the Austrian Agency for Health and Food Security (AGES) in Vienna) took part in this exercise. A number of well characterised samples (four positives and four negatives) were sent as blind samples to each laboratory and the participants were asked to determine the diagnostic status of these samples. The results were collected, analysed and presented in December 2012, first at the PPR CRP meeting in Vienna and subsequently at the West and Central Africa Veterinary Laboratory Network (RESOLAB) meeting in Dakar, Senegal. Only six counterparts had 100% correct results (Table 2). Those who failed one or more tests were contacted with questionnaires in order to help them improving their testing. Another proficiency test will be organized in 2013.

TABLE 2: SUMMARY RESULTS OF THE 2012 PPR RING TEST
(*LABS. 5 AND 7 – *RESULTS YET TO BE RECEIVED)*

	Positive	Negative	False Positive	False Negative	Total number of Samples
Lab 1	4	2	2	0	8
Lab 2	4	0	4	0	8
Lab 3	4	0	4	0	8
Lab 4	3	4	0	1	8
Lab 5*					8
Lab 6	**4**	**4**	**0**	**0**	**8**
Lab 7*					8
Lab 9	4	3	1	0	8
Lab 10	**4**	**4**	**0**	**0**	**8**
Lab 11	4	2	2	0	8
Lab 12	3	3	1	1	8
Lab 13	**4**	**4**	**0**	**0**	**8**
Lab 14	2	2	2	2	8
Lab 15	**4**	**4**	**0**	**0**	**8**
Lab 16	4	3	1	0	8
Lab 17	4	2	2	0	8
Lab 18	4	1	3	0	8
Lab 19	**4**	**4**	**0**	**0**	**8**
Lab 22	**4**	**4**	**0**	**0**	**8**
Lab 23	3	2	2	1	8

Legend: Positive; False Positive; Negative; False Negative; 100% Correctness

Serological diagnosis of Rift Valley fever (RVF)

Another proficiency test was organized by APHL in 2012 in collaboration with the Special Pathogens Unit at the National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service in Johannesburg, South Africa, on the serological diagnosis of RVF. RVF is an acute or sub-acute viral disease of many ruminants, but particularly severe in sheep. It is transmitted by many blood-sucking insects. It is a major zoonosis, i.e. a serious disease of humans who are usually infected by contaminated livestock and livestock products. The ring test formed part of the activities of the "Identify" project and was implemented in close collaboration with the FAO Animal Health Service and the FAO Emergency Centre for

Transboundary Animal Diseases (ECTAD) regional units for East Africa and for West and Central Africa. The objective was to put in place a valid external quality assurance platform for the serological testing of RVF. It is planned to repeat this exercise at least twice following on this initial ring test. RVF competitive ELISA kits from two different manufacturers were submitted to the proficiency test and provided, together with the proficiency panels, to participating laboratories. Under a technical contract, the Special Pathogen Unit of the NICD prepared and supplied the panels. Evaluation and the provision of feedback are currently in progress. The following countries were involved: Cameroon, Central African Republic, Congo, Democratic Republic of Congo, Ethiopia, Kenya, Rwanda, Senegal, Tanzania and Uganda.

Meetings

A consultant meeting on *"Applying Good Laboratory Practices in Molecular Testing of Multiple Diseases in Veterinary Laboratories"* was held in IAEA, Vienna on 16-18 October 2012. The meeting discussed the applications of appropriate molecular diagnostic technologies and platforms suitable for veterinary testing laboratories with minimum resources, recommendations on practical implementation of GLP conditions, the multidisciplinary approach, core facility and sustainability in veterinary diagnostic and testing laboratories of Member States. Experts from Member State laboratories discussed the current status of molecular tools being implemented and the further measures to be taken for successful and better disease management. Experts from the National Veterinary Institute of the Swedish University of Agricultural Sciences (Uppsala, Sweden), the CIRAD-INRA French Initiative for International Agricultural Research (IFRAI; France), the Institute of Tropical Medicine Antwerp (Belgium), the Animal Health and Veterinary Laboratories Agency (Surrey, UK), the Laboratoire de Virologie of the National Laboratory for Veterinary Research at the Senegalese Agricultural Research Institute (Dakar, Senegal), the Instituto Zooprofilattico Sperimentale delle Venezie (Italy), the CSIRO Livestock Industries (Australia), the National Centre for Veterinary Diagnostics (Vietnam), the National Institute of Animal Health (Thailand) and the FAO (Rome, Italy) discussed the strategic guidelines needed to be implemented in molecular testing laboratories of Member States.

Capacity building

In 2012, the APHL hosed one cost-free expert (CFE), four fellows and three interns in the following areas:

Name	Country	Status	Duration	Topic
Clarke, Matthew	UK	Intern	2 mth	PPR diagnosis and molecular epidemiology of the disease
Galanxhi, Xhiljola	Albania	Intern	7 mth	Diagnosis of PPR by gene amplification. She was involved in the development of new diagnostic tests

Name	Country	Status	Duration	Topic
Shuo, Li	China	Intern	6 mth	PPR diagnosis and molecular epidemiological study of the disease
Chibssa, Tesfaye Rufael	Ethiopia	Fellow	4 mth	Molecular diagnosis of PPR and capripox
Boldbaatar, Chinchuluun	Mongolia	Fellow	2 mth	Advanced molecular diagnostic techniques; experimental design and implementation
Arinaitwe, Eugene	Uganda	Fellow	3 mth	PR diagnosis: virus isolation, nucleic acid amplification and gene sequencing
Ralambomana, Norbertin	Madagascar	Fellow	2 mth	Molecular genetic tools
Xu, Wu	Fujian Agriculture and Forestry University, China	CFE	8 mth	Molecular characterization of indigenous sheep breeds and radiation hybrid panel mapping of goat genome

PUBLICATIONS

Diallo, A., Libeau, G. (in press). Peste des petits ruminants. In: Liu, D. (Ed.). Manual of Security Sensitive Microbes and Toxins. CRC Press.

Kamissoko, B., Sidibé, C.A.K., Niang, M., Samaké, K., Traoré, A., Diakité, A., Sangaré, O., Diallo, A., Libeau, G (in press). Prévalence sérologique de la peste des petits ruminants (PPR) des ovins et caprins à travers le Mali (Seroprevalence of peste des petits ruminants (PPR) in sheep and goats flocks in Mali). Rev. Med. Pays Tropicaus.

Bionaz, M., Kathiravan, P., Rodriguez-Zas, S.L., Hurley, W.L., Loor, J.J. (2012). A novel dynamic impact approach (DIA) for functional analysis of time course Omics studies: Validation using the bovine mammary transcriptome. PLoS ONE 7(3): e32455. doi:10.1371/journal.pone.0032455.

Bionaz, M., Kathiravan, P., Rodriguez-Zas, S.L., Everts, R.E., Lewin, H.A., Hurley, W.L., Loor, J.J. (2012). Old and New Stories: Revelations from functional analysis of the bovine mammary transcriptome using the lactation cycle. PLoS ONE 7(3): e33268. doi:10.1371/journal.pone.0033268.

Kathiravan, P., Kataria, R.S., Mishra, B.P. (2012). Power of exclusion of 19 microsatellite markers for parentage testing in river buffalo (*Bubalus bubalis*). Mol Biol Rep, 39(8):8217–23.

Dubey, P.K., Aggarwal, J., Goyal, S., Gahlawat, S.K., Kathiravan, P., Mishra B.P., Kataria, R.S. (2012). Sequence and topological characterization of Toll-like receptor 8 gene of Indian riverine buffalo (Bubalus bubalis). Trop Anim Health Prod 45(1):91-9.

Ganapathi, P., Rajendran R., Kathiravan, P. (2012). Detection of occurrence of a recent genetic bottleneck event in Indian hill cattle breed Bargur using microsatellite markers. Trop Anim Health Prod 44(8):2007-13.

EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
Centre de Coopération Internationale pour la Recherche Agronomique et le Développement (CIRAD), France	PPR and capripox research
The Pirbright Institute, UK	Capripox research
National Animal Health Diagnostic and Investigation Center (NAHDIC), Ethiopia	Capripox research
National Veterinary Institute (NVI), Ethiopia	Capripox research
Pan African Veterinary Vaccine Centre (PANVAC), Ethiopia	Transfer of animal disease diagnostic test; improvement of vaccine quality
Laboratoire Central Vétérinaire (LCV), Mali	Capripox and PPR research
High Security Laboratory, Institute for Veterinary Disease Control, Austrian Agency for Health and Food Security (AGES), Mödling, Austria	Exotic animal diseases research (Capripox, PPR, ASF)
Laboratoire National Vétérinaire (LANAVET), Cameroon	ASF
Special Pathogens Unit of the National Institute for Communicable Diseases, South Africa	RVF
Laboratoire National d'Elevage et de Recherches Vétérinaires (LNERV/ISRA), Senegal	Capripox, PPR, ASF

EXTRABUDGETARY SUPPORT

In addition to IAEA regular budget and TC projects, activities APHL activities were supported by the following projects:

IDENTIFY PROJECT: Support for strengthening animal health laboratory capacities in hot spot regions to combat zoonotic diseases that pose a significant public health threat. Tripartite FAO/OIE/WHO, funded by the United States Agency for International Development (USAID)

AFRICAN RENNAISANCE FUND (ARF): Improvement of veterinary laboratory capacities in South Saharan African countries. Funded by the Department of International Relation and Cooperation of the Republic of South Africa.

PEACEFUL USES INITIATIVE (PUI): The improvement and capacity building of nuclear and nuclear related animal disease diagnostic capacities of veterinary laboratories at the regional level in Africa and Asia. Funded by the United States Department of State.

THE FOOD AND ENVIRONMENTAL PROTECTION LABORATORY

EXECUTIVE SUMMARY

Food safety and quality are vital aspects of food security. Our food is vulnerable to a range of food hazards (microbiological, chemical and physical) that may arise at any stage of the food supply chain. Increasing volumes of global trade and the growing complexity of supply chains over recent decades have added to concerns regarding food safety, quality and authenticity. In addition to well publicized food safety incidents such as aflatoxins in maize, dioxins in pork, melamine in dairy products, and Salmonella in peanuts, new hazards and risks are continually emerging. These may be related to contamination with, e.g., microbes, food additives or residues of agrochemicals, or intentional adulteration for economic fraud or with the intent to harm consumers. Commodities that attract premium prices or are traded in high volume run the risk of being subject to fraud such as adulteration or counterfeit, and this can pose risks due to the unknown origin of the substances added to, or substituted for, the authentic product. Other issues may also have an impact on food safety although the issues might not yet be well understood or characterized, for example the effects of climate change on food production, or emerging technologies such as the use of nanoparticles in food and packaging.

The FEPL advocates a holistic, farm-to-fork approach for effective systems to help ensure food safety, quality and security. Member State laboratories are the chief direct recipients of the FEPL outputs, but such systems must involve stakeholders at all points along the food supply chain for effectiveness and long term viability. Our approach is to enhance the capabilities of analytical laboratories and to encourage interactions between the laboratories and multiple stakeholders, thereby providing essential feedback and advice to help build the capacity to assess and manage risks and improve agricultural practices. Direct contributions from FEPL include the development and transfer of methodologies for food traceability, food authenticity, and for the control of chemical contaminants, the development and provision of technical protocols, advice and guidance, and inputs for the development of international standards.

Research and development achievements in 2012 included the development of methodologies for food traceability and authentication. These focused on honey and fruit juice products, both of which are important commodities in international trade and frequent targets for fraudulent practices such as counterfeiting or adulteration. Methodology established to facilitate the stable isotope analysis of honey included a method for the extraction and purification of protein from honey, a dual equilibration method for proteins and organic samples, and a procedure to replace exchangeable hydrogen atoms in carbohydrates with nitrate functional groups to allow direct measurement of non-exchangeable hydrogen isotopes. A metabolomics approach for the assessment of fruit juice authenticity using time of flight mass spectrometry was developed and shown to be a promising method for detecting adulteration of high value juices with lower value substitutes. To support this field of work, a number of candidate certified reference materials for light stable isotope analysis were identified for further validation. A consultants' meeting was convened to develop a new Coordinated Research Project, to commence in 2013, on accessible technologies for the verification of origin of dairy products as an example control system to enhance global trade and food safety.

Achievements related to the control of residues and contaminants in food include the development of multi-residue analytical methods for the determination of residues of veterinary antimicrobials in meat, and for pesticides in carrot and fish. Protocols for pesticide toxicity tests and bioassays to screen for contamination were developed, validated and disseminated. Work was continued in an inter-Agency project that aims to establish capacity for the quality control of veterinary trypanocidal drugs in sub-Saharan Africa; outputs of international significance include eight monographs, submitted for publication by OIE as the first step in their acceptance as international standards. Analytical methods, Standard Operating Procedures (SOPs) and a training programme were also developed for reference laboratories in Africa, adding to comprehensive support for work in this part of the world.

The FEPL was involved in an advisory capacity in an EU project focusing on the development of inexpensive methods for the detection and control of contaminants in food and feed. The project was completed in 2012, with the development and validation of a number of cheap, robust, multiple contaminant methods, some of which are suitable for transfer to IAEA Member State laboratories.

Outreach activities included the presentation of the results of FEPL research at six international conferences, and the FEPL was involved directly in the scientific committees for two major international conferences. The FEPL also presented its work on food traceability and contaminant control in the final session, “Enhancing Food Safety”, of the 2012 IAEA Scientific Forum, “Food for the Future - Meeting the Challenges with Nuclear Applications” held during the 56th IAEA General Conference.

Capacity building activities in 2012 included the technical management of eighteen national and seven regional Technical Cooperation Projects. The expertise available in the FEPL and the methods and techniques developed were also used to support technology transfer to Member States. Two train-the-trainers workshops at Seibersdorf and three regional workshops in Member States were held with extra-budgetary funding, with more than ninety developing country scientists being trained in various aspects of food safety control. The FEPL hosted three individual fellowships and group training for six Scientific Visitors, totalling approximately eleven man-months. Two internships were also completed, and one cost-free expert gained knowledge and experience whilst assisting in the implementation of FEPL activities. A sustainable, formal network of sixteen laboratories in Latin America and the Caribbean, the Red Analitica de Latino America y El Caribe (RALACA), was initiated and established with FEPL assistance to promote and support food safety and environmental sustainability.

Publications by FEPL staff included five papers in the peer-reviewed scientific literature, nine papers in conference proceedings/books of abstracts or meeting reports, and one book chapter.

STAFF

Name	Title
Cannavan, Andrew	Laboratory Head
Frew, Russell David	Food Safety Specialist
Maestroni, Britt Marianna	Food Scientist
Jandrić, Zora	Analytical Chemist
Rathor, Mohammad Nasir	Laboratory Technician
Islam, Marivil	Laboratory Technician
Abrahim, Aiman	Laboratory Technician
Wimberger, Tamara	Team Assistant

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Food traceability and authenticity

Questions concerning origin are among the first to be asked when a food safety incident arises. In addition, consumers in key markets are increasingly concerned with the origin of their food and are willing to pay more if they can be assured of its origin.

One of the primary tools for ensuring food safety is a traceability system. These are paper-based systems that pass information along with the commodity, giving both industry and the consumer confidence that the product they are purchasing comes from a supplier with the appropriate food safety and quality measures in place. All such systems are subject to failure either inadvertently or deliberately (fraud). The incidence of fraud is difficult to measure but estimates from a recently completed EU project suggest levels of 15 – 20% are likely in the European market, and this may be higher elsewhere.

It is recognized that there is a need for a system to verify the origin of food and hence audit traceability control systems. Nuclear techniques are proving to be very effective in authenticating food products (i.e. detection of adulteration or counterfeit), and in discriminating between foods from different geographical origins. These analytical techniques have the potential to verify information-based traceability systems and to provide information on the integrity of the food product.

In 2012, the FEPL performed research and method development in this field in parallel with the current Coordinated Research Project (CRP) on the “Implementation of Nuclear Techniques to Improve Food Traceability” for future technology transfer. Research focused on two high value commodities that are important in global trade and are common targets for deliberate adulteration; honey and fruit juice.

Authenticity assessment of honey

Fraud is a major issue in the food industry. Challenges to authenticity range from defrauding an established brand by representing a cheaper product through relabeling to gain the price advantage of the higher valued product to activities such as addition of cheaper components to "stretch" a product. In addition to their economic implications, these activities carry serious food safety risks, since the inferior products are unlikely to have been subject to the same stringent quality controls as the genuine article. They are a lost sale to the genuine producer and may result in loss of brand value and market access. The FEPL is developing a research programme that applies nuclear techniques for verification of the authenticity of foodstuffs. The tools being developed will be applicable to the detection of adulteration and/or the verification of the origin of a food in both developing and developed countries.

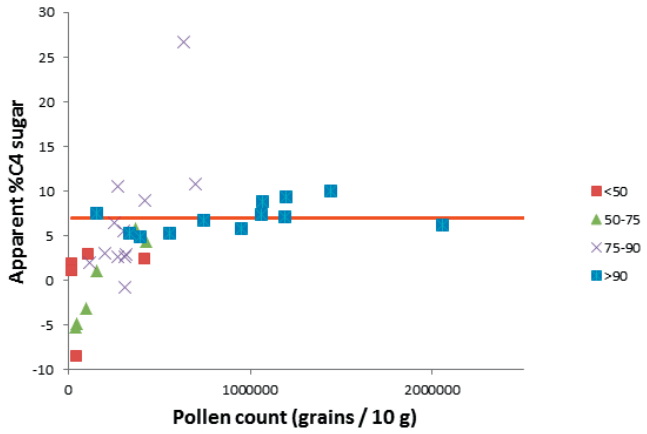

FIG 1. Pollen count versus apparent %HFCS adulteration using the standard test. The horizontal red line indicates the cut-off point of 7% above which the honey is deemed to be adulterated. The samples are classed into groups according to the percentage of the total pollen that was found to be Manuka, this is represented in the legend.

Honey is a food that has been an integral part of most cultures for centuries. It has also been subject to fraud for almost as long; the most common fraud being the "stretching" of genuine honey with cheaper sugars. More recently, trade in honey has become a major issue, with several high-profile economically-driven incidents involving honey being transhipped through other countries to avoid tariffs.

Stable isotope techniques have been used for over a decade to detect the addition of high fructose corn syrup (HFCS) to honey. The technique involves extracting the protein from the honey and comparing the carbon isotopic composition with that of the carbohydrate; the presence of HFCS will show up as a discrepancy between these two values (Fig. 1). While this technique has served the industry well for several years there are two issues that need to be addressed;

1) There is a high failure rate of the test among the very highest value honeys such as the New Zealand Manuka (>70% pollen count is classed as Manuka - blue squares and crosses in Fig. 1).

2) Fraudsters are now using rice syrup to stretch the honey. Rice is identical to honey in the carbon isotopes and hence indistinguishable by the carbon isotope technique.

The approach at FEPL is to develop a similar technique but utilising the hydrogen isotopes. The carbon isotopic ratios are primarily determined by the plant type (photosynthetic pathway) while the hydrogen isotopes vary geographically in patterns similar to those found in the

isotopic composition of the rainfall. The complication with using hydrogen is that a significant portion of the hydrogen in natural samples is able to exchange with that in ambient water and so a method that enables the measurement of the non-exchangeable hydrogen is required.

The main technical developments for this work achieved in 2012 include;

1) Implementation of a method for the extraction and purification of protein from honey

2) Implementation of a dual equilibration method for protein and other organic samples that allows the effect of exchangeable hydrogen to be accounted for by mass balance

3) The development of an analytical procedure to remove all exchangeable hydrogen atoms from carbohydrate and replace these with nitrate functional groups, thus allowing the direct measurement of the non-exchangeable hydrogen isotopes.

Development of new certified reference materials

One of the limitations of the stable isotope technique is its reliance on suitable certified reference materials (CRMs). There has been an enormous expansion in techniques and applications, but the availability of CRMs, particularly for foodstuffs, has not kept pace. The IAEA's Terrestrial Environmental Laboratory (TEL) has a large catalogue of CRMs that have been developed for other purposes such as radionuclides or trace metals. The FEPL and TEL have initiated a collaborative programme to investigate the suitability of some of these materials for development into CRMs for light isotope analysis, for use in food commodities. The initial survey work was completed in 2012 and several materials appear to fulfil the initial criteria of being a food-type matrix and having a wide range in stable isotope composition (Table 1). Work is continuing to verify that these potential CRMs meet the more stringent criteria of stability and homogeneity.

TABLE 1. CARBON AND NITROGEN ISOTOPE RESULTS FROM THE FIRST SURVEY OF SEVERAL IAEA CRMS

CRM Designation	$\delta^{15}N_{AIR}$‰	$\delta^{13}C_{PDB}$‰	N, % by mass	C, % by mass	n
IAEA 152: AMP	5.83 ± 0.19	-25.03 ±0.05	5.57 ± 0.05	41.31 ± 0.25	18
IAEA 156: Clover	1.03 ± 0.24	-27.11 ± 0.23	2.41 ± 0.10	38.83 ± 0.41	18
IAEA 330: Spinach	4.76 ± 0.17	-28.10 ± 0.11	5.15 ± 0.05	42.14 ± 0.34	24
IAEA 336: Lichen	-13.46 ± 0.46	-22.65 ± 0.09	0.59 ± 0.02	42.23 ± 0.47	30
IAEA 359: Cabbage	18.64 ± 0.21	-25.74 ± 0.08	4022 ± 0.02	39.26 ± 0.17	18
IAEA 372: Grass	2.26 ± 0.20	-27.53 ± 0.11	4.69 ± 0.11	44.37 ± 0.23	18
IAEA 373: Grass	0.82 ± 0.34	-27.18 ± 0.14	1.74 ± 0.11	45.32 ± 0.58	18
IAEA 413: Algae	2.42 ± 0.10	-39.80 ± 0.26	9.73 ± 0.08	46.77 ± 0.43	18
IAEA 447: Moss Soil	-2.48 ± 0.21	-20.56 ± 0.24	1.03 ± 0.02	15.39 ± 0.49	18
IAEA MA-A-1: Copepod	8.10 ± 0.18	-22.83 ± 0.21	7.92 ± 0.16	44.30 ± 0.61	18

CRM Designation	$\delta^{15}N_{AIR}$‰	$\delta^{13}C_{PDB}$‰	N, % by mass	C, % by mass	n
IAEA V-8: Rye Flour	4.25 ± 0.42	-23.18 ± 0.11	0.59 ± 0.01	39.81 ± 0.21	18
IAEA V-9: Cotton Cellulose	-	-24.47 ± 0.09	<0.01	41.75 ± 0.20	18

Assessment of fruit juice authenticity using UPLC/QToF MS: A metabolomics approach

A highly prized food commodity popular as a target for adulteration and fraud is fruit juice. Fruit juice commodities where in the top seven foods reported as the most common targets for adulteration from 1980 to 2010. Fruit juice products are consumed worldwide, and have become very popular in recent years, due in part to their perceived health benefits.

The most common fruit juice adulteration practices are dilution with water and addition of sugars, pulp wash, or other, cheaper, fruit juice. The magnitude of the fraud is estimated to be up to US $37 million annually in the USA alone. Fruit juice fraud is often considered to be mainly an economic issue and less a concern from a food safety point of view. However, the nutritional value of fruit juices may be degraded with adulteration and some substituted ingredients can have direct health impacts; for example, they may cause allergic reactions.

Detecting adulteration can be a complex task due to the natural variation in fruit cultivars, as well as differences that occur with different growing regions, storage conditions, sample treatment and processing techniques. One of the most difficult types of fruit juice adulteration to detect is the addition of inexpensive juice to a premium value product. The methods used most frequently to analyse for this type of fraud involve profiling and/or targeted analysis of a group or combination of specific compounds. Disadvantages of these methods are that usually only one type of adulteration can be detected, and the cost is high.

A new discipline used to explore and characterize the complexity of biological pathways, metabolomics, has been shown to be of great promise for food fingerprinting. Thousands of metabolites can be found in plants that could potentially be markers for food authenticity assessment. A study was undertaken in the FEPL to discover novel biomarkers for detection of the adulteration of two high value fruit juices (pineapple and orange) with cheaper alternatives (apple, grapefruit and clementine). Each fruit juice has a typical metabolomic fingerprint and pattern that can be used as a signature for the authentic product. Research therefore focused on assessing fruit juice authenticity by examining components of these fingerprints using ultra-performance liquid chromatography – quadrupole time-of-flight mass spectroscopy (UPLC-QToF MS) coupled with multivariate statistical analysis.

Authentic and adulterated fruit juice samples (pineapple, orange, grapefruit, apple and clementine) were prepared in the laboratory. Untargeted metabolite fingerprinting was performed by UPLC-QToF MS and multivariate data analysis. Twenty differential metabolites were detected that contributed to the clear separation between pineapple and orange and their admixtures at 5% adulteration level (Fig. 2). A reliable, rapid, sensitive and robust method targeting selected markers was then optimized for confirmation purposes and it was possible to detect adulteration at a level of 2%, typical of the low levels used in fraudulent practice.

Future work will further validate the methodology by building up a database of the metabolic patterns of many different fruit varieties.

The results demonstrate that metabolomics has potential as a screening tool for the detection of food fraud by adulteration, and could represent a new strategy in food forensics to enable a rapid response in the global fruit juice market to help regulators to stay one step ahead of fraudsters.

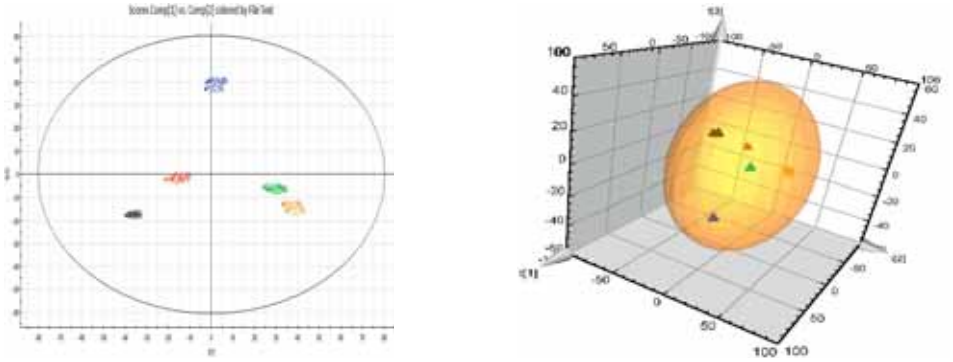

FIG. 2. The PCA scores plot and 3-D plot showing clear segregation between the sample groups (pineapple and pineapple mixed with orange, grapefruit, apple, and clementine) at 5% adulteration level.

Isotopic traceability techniques for rapid response to emerging food safety risks

A consultants' meeting was held at IAEA headquarters in Vienna, Austria from 11–14 September 2012. Five internationally recognised experts participated. The state of the art for determining the point of origin and integrity of food was discussed at length. There has been considerable investment in the science, but despite many studies that demonstrate its usefulness, there has been very limited uptake of this technology to date. The reasons for this include:

- The high cost to entry, as a lot of background information needs to be collected using expensive (and often slow) analytical techniques.
- The interpretation of the data and the level of certainty attainable have been hampered by the limited availability of reference data and the lack of standardized, robust and accessible multi-variate and spatial data analysis tools.
- Lack of awareness; most of the stakeholders, including regulators and producers, are unaware of the capabilities of this technology and so it has not gained widespread acceptance.
- The current bespoke nature of the technology requires a high level of expertise for implementation.

Recent developments in instrumentation (e.g. laser-based analysers for light isotopes and trace metals) reduce analytical costs considerably and may facilitate accessible systems and uptake. However, the new technologies need to be assessed to determine their fitness for purpose.

The meeting resulted in a CRP proposal, "Accessible Technologies for the Verification of Origin of Dairy Products as an Example Control System to Enhance Global Trade and Food Safety", which was subsequently approved and will commence in 2013. The example

system developed will serve as a template for other commodities and the technology will be transferrable. The CRP will generate the data coverage to enable the appropriate statistical and mapping tools to be applied and refined, and will also raise awareness of what the technology can offer, especially to policy-makers and end users. Ultimately the users of this technology will be food producers, regulators and policy-makers within Member States.

Control of residues and contaminants in food

A number of analytical methods and protocols were developed and validated in the FEPL and transferred to Member States through training at Seibersdorf, in Member States or through CRPs. In addition to the methods summarized below, protocols were developed for non-isotopic screening and complimentary methods for use in Member States as components of analytical method packages to allow the cost-effective implementation of contaminant control strategies. These included protocols for the evaluation of pesticide toxicity and for biomonitoring in water courses as a first tier screening tool to indicate environmental contamination. This integrated approach uses simple and cheap screening methods to identify potential contamination problems, allowing the more targeted and cost-effective application of isotopic and related techniques to further characterize the contamination detected. The information can then be used to improve agricultural practices and optimize both food safety and environmental sustainability.

A liquid chromatography-tandem mass spectrometry method for antibiotic residues in meat

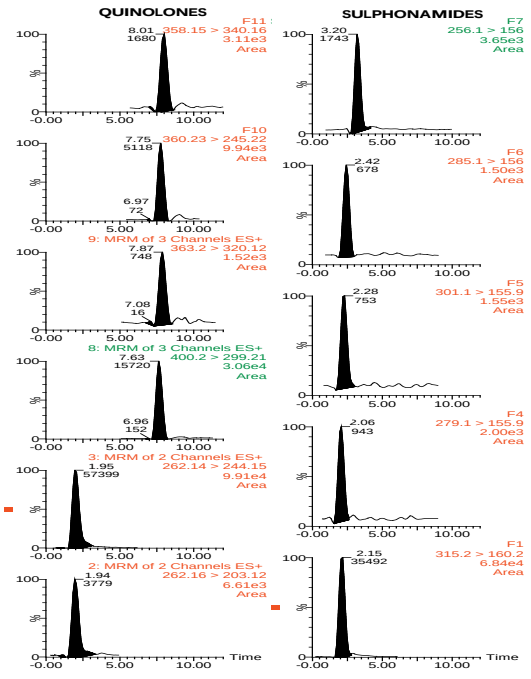

FIG. 3. Chromatograms of six quinolones and four sulphonamides at 0.25 × MRL, including the internal standard, sulphaphenazole.

Antibiotics are extensively used to fight infectious diseases in both humans and animals. In recent years, several cases have been reported of antibiotic resistant bacteria which have been transmitted from animals to human either through direct contact or after ingesting animal-derived foods (meat, fish, milk and milk products). Quinolones are a class of antimicrobials critically important in the treatment of severe and aggressive infections in both humans and animals and are therefore of special interest for public and animal health. The use of the fluoroquinolone drug, enrofloxacin, in animal production has been linked with the development of strains of Salmonella spp. and Campylobacter spp. resistant to ciprofloxacin, a related drug used in human treatment. Similar problems have been documented on the widespread resistance of some animal pathogens to sulphonamides, which are synthetic antimicrobial agents widely used to treat bacterial diseases in animals. Quinolones and sulphonamide antibiotics are therefore of high concern to regulatory authorities, since they may be found as residues in food from treated animals and pose risks not only through their potential toxicity if ingested,

but also through the development of antibiotic resistant pathogens. The presence of these drugs in food must be monitored and controlled to protect human health.

A fast and simple multiclass method for the determination of six quinolone drugs (flumequine, oxolinic acid, difloxacin, marbofloxacin, enrofloxacin and danofloxacin) and four sulphonamides (sulphamethazine, sulphaquinoxaline, sulphachloropyridazine, and sulphathiazole) in porcine meat was developed and validated in the FEPL. A simple extraction method was employed for porcine kidney using 2% trichloroacetic acid/acetonitrile solution, with a simple hexane wash clean-up. The extract was diluted with formic acid/acetonitrile, filtered and analysed by liquid chromatography tandem mass spectrometry. Separation of the compounds was achieved using a HILIC analytical column with gradient elution and a run time of 12.5 min. The method was validated with reference to the maximum residue limits (MRLs) set by the European Commission, which range between 150 – 1500 µg/kg for the targeted quinolones and are set at 100 µg/kg for the sum of the sulphonamides found in a sample. Method recoveries ranged from 78 to126% with reproducibility values ranging from 15.3 to 29.4%.

Antibiotic abuse in animal production is especially critical in developing countries. This method was developed as part of the training programme for a TC Fellow (TC project BOT/5/006) and transferred to a laboratory in Botswana to help establish the capacity for veterinary drug residue monitoring in food of animal origin to protect public health and enhance international trade.

A multi-residue method for the analysis of contaminants in carrot and fish using a modified QuEChERS clean-up and GC-MS detection

Pesticides are widely used in agriculture, especially in warm-tropical climates, to protect crops that are prone to infestation by various pests and diseases. Some agrochemicals now obsolete in many developed countries may still be in use by farmers in developing countries because of the lack of regulatory enforcement or due to a lack of knowledge and awareness on the toxicity of the pesticides and their mobility in the environment. Residues of these pesticides can remain on the crops and therefore it is necessary to have analytical methods that can detect and quantify their presence on the crop. Analytical laboratories must be able to test a wide range of food matrices for a large number of pesticides.

A sample preparation protocol using a modified QuEChERS (Quick, Easy, Cheap, Efficient, Reliable, Safe) method was optimized in the FEPL for a total of 29 pesticides in carrot and fish matrices, with detection by gas chromatography coupled to a single quadrupole mass spectrometer (GC-MSD). The method was optimized in the range 0.05-5 mg/kg. Samples were finely chopped in a homogenizer and extracted by homogenising with ethyl acetate together with a mixture of sodium hydrogen carbonate and sodium sulphate. The extracts were cleaned up by shaking with primary-secondary amine, C18 reversed-phase chromatographic material and magnesium sulphate. The run time for the method was 35 minutes and typical target analyte recovery was in the range 79-106%, which is in accordance with the Codex Alimentarius acceptable range (70-120%) as a measure for accuracy. Fig. 1 shows a typical chromatogram for nineteen of the target pesticides at highest fortification level in carrot; chromatograms for fish were similar.

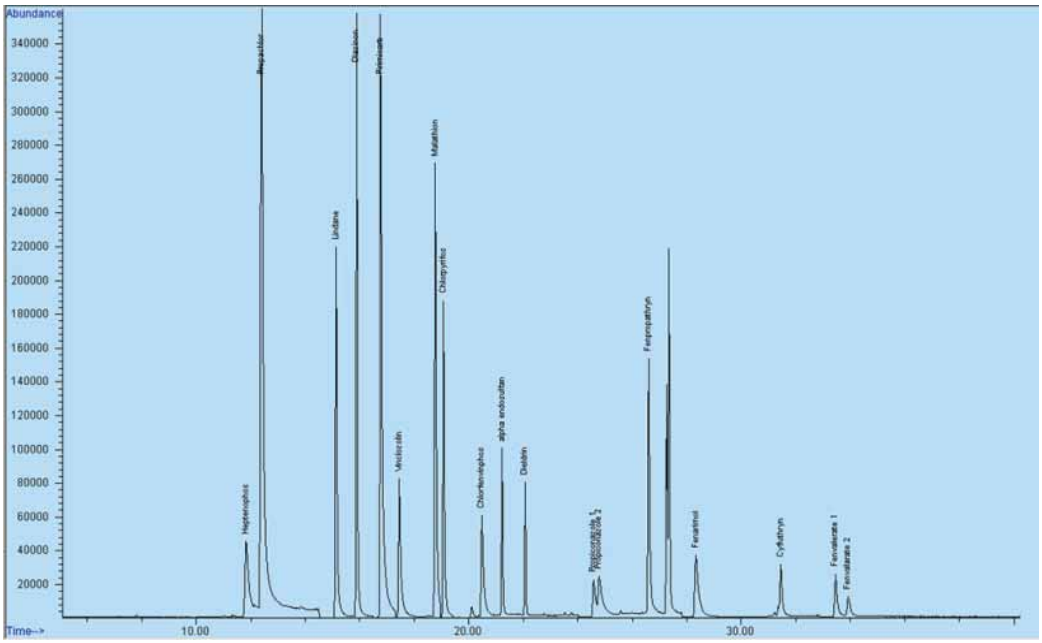

FIG. 4. Chromatogram of nineteen of the target pesticides at the highest fortification level in carrot

The method was demonstrated at a training workshop in October 2012 to fifteen participants from nine developing countries. The method is quick and relatively cheap and can be adapted to cover a number of old or obsolete GC-amenable agrochemical compounds in both developed and developing country regulatory laboratories.

Quality control of trypanocidal drugs

Tsetse-transmitted African animal trypanosomosis is arguably the most important animal disease impairing livestock agricultural development in sub-Saharan Africa. Besides vector control, the use of trypanocidal drugs is the main method to control the impact of the disease on animal health and production in most sub-Saharan African countries. Presently there are three compounds widely available to treat trypanosomosis; diminazene aceturate, isometamidium chloride hydrochloride and homidium (chloride and bromide salts).

Various studies and market surveys on the quality of the various trypanocidal pharmaceutical formulations sold in different markets in sub-Saharan Africa have shown that a substantial proportion of the products were of sub-standard quality or even fake, containing no active trypanocidal substance. This has severe implications for animal health, public health and the local economy. Inappropriate treatment results in animal morbidity or mortality and increases the risk of the emergence of drug resistance in trypanosome populations when animals are treated with medications that contain a lower amount of active ingredient than the recommended dosage; there are currently 17 African countries in which animal trypanocidal drug resistance has been reported. Moreover, food safety is compromised by allowing unspecified and potentially harmful chemicals to enter the food chain. Effective quality control of the drug formulations is hampered by the lack of internationally agreed standards or specifications.

In line with the recommendation of the OIE Conference on Veterinary Medicinal Products in Africa, Dakar, Senegal, 2008, and in consultation with African institutions, an international alliance was created to help address this problem. The alliance includes FAO, IFAH, GALVmed, IAEA, the University of Strathclyde and Manchester Metropolitan University. The alliance developed pharmacopoeia-type monographs for isometamidium chloride hydrochloride, diminazene diaceturate, homidium chloride and homidium bromide and their formulations. The monographs have been submitted for publication by OIE. Standardized analytical procedures for evaluating the quality of the drugs were elaborated and cross-validated in the University of Strathclyde and the FEPL.

The analytical procedures developed are being transferred to two laboratories in sub-Saharan Africa. These laboratories will form the basis of a system to enable reliable quality control by drug registration authorities. A programme was developed in 2012 in the FEPL in collaboration with Manchester Metropolitan University for the training of laboratory analysts on the chromatographic analytical methods.

The monographs developed through this alliance and the supporting analytical methodology will ultimately allow laboratories in Africa, Asia and South America, as well as those of veterinary pharmaceutical companies, to carry out the quality control of the described trypanocidal drugs on a common platform.

EU 7th Framework Project "CONffIDENCE"

The EU 7th Framework Integrated Project "Contaminants in Food and Feed: Inexpensive Detection for Control of Exposure" (CONffIDENCE) was completed in December 2012. The project included seventeen partners from ten countries and a budget of €7.5 million, of which €5.8 million is from the EC. The final meeting of the CONffIDENCE consortium, Project Management Board, and Advisory Board took place in Brussels, Belgium, on 17-18 December 2013. Mr Cannavan, Head of the FEPL, participated in the project as Chair of the Advisory Board.

The goal of the CONffIDENCE project was to develop relatively fast and cheap methods to protect consumers from the effects of a wide range of potentially harmful chemical residues in food and feed. This was achieved: a number of field/screening methods and laboratory based methods were developed, validated and are being implemented by industry and regulatory authorities. Some of the rapid, simple screening tests produced are relevant to problems in countries involved in IAEA Technical Cooperation Projects (TCPs) and will be transferable through the TCP mechanism. The involvement of IAEA in the CONffIDENCE Advisory Board was important in ensuring that such methods were developed and validated with a wider perspective than the European market, and will be of benefit to developing countries globally.

Further information on the project can be found at *http://www.conffidence.eu/*.

Dissemination of research results

The methods developed, refined and validated in the FEPL are made available to Member States through various mechanisms, including training courses and publications in the scientific literature. In 2012, a new database developed by the Food and Environmental Protection Subprogramme was made available as an online resource. The Food Contaminant and Residue Information System (FCRIS, *http://nucleus.iaea.org/fcris/*) provides a wealth of useful data on food contaminants and also hosts methods databases for veterinary drug residues and for pesticide residues, developed in response to requests from the Codex Committees on Residues of Veterinary Drugs in Food and on Pesticide Residues. These databases include methods developed in the FEPL, as well as others submitted by laboratories in Member States.

FEPL staff participated in, and presented research and development results at, several international conferences and seminars in 2012, including:

- An oral presentation and two posters at the EuroResidue VII Conference on Residues of Veterinary Drugs in Food, Egmond aan Zee, the Netherlands, 14-16 May 2012. Approximately three hundred and thirty scientists from thirty seven countries throughout the world participated in EuroResidue VII. Two posters from the FEPL were presented, one on methodology developed in the FEPL for a multi-class liquid chromatography-tandem mass spectrometry method for anthelmintics in soil and water and the second on the establishment of national residue control programmes for antibiotics and anthelmintic veterinary drug residues in developing countries. An oral presentation on regional approaches for the control of veterinary drug residues in developing countries was also given in a conference workshop on global trade and stakeholders.
- Presentation of a poster at the 1st European Workshop on Ambient Mass Spectrometry and Related Mass Spectrometry-based Techniques in Food/Natural Products Control: Safety, Authenticity, Forensics, Metabolomics, held in Prague, Czech Republic, 18-20 June 2012. The workshop had approximately eighty participants. A poster entitled "Assessment of Fruit Juice Authenticity Using UPLC/QToF MS and MarkerLynx Data Evaluation", was presented, which summarised the outcome of research performed in FEPL under the programmatic field "Traceability to improve food safety and quality and enhance international trade".
- Presentation of a poster at the 4th International Feed Safety Conference - Methods and Challenges, held in Beijing, the People's Republic of China, 11-13 September 2012. The conference was attended by more than three hundred participants from more than twenty five countries. A poster was presented on the development and application of a method for the simultaneous determination of natural toxins in animal feeds by liquid chromatography-tandem mass spectrometry. The research was carried out to provide analytical methodology for the risk assessment of feed contamination and carry-over of natural toxins from feed to food.
- A poster presentation at the European Pesticide Residues Workshop, which took place in Vienna from 25-28 June 2012. More than five hundred and twenty participants from fifty four countries attended the conference, which is the leading international meeting in this field. The poster summarised a five year international CRP on integrated analytical approaches to assess indicators of the effectiveness of pesticide

management practices at a catchment scale. This CRP, coordinated and supported by the FEPL, brought together analytical laboratories in a network to apply harmonized and integrated approaches to monitor the presence of selected high-impact-ranking pesticides in surface water and sediments.

- A poster presentation at the international conference on "Worlds Within Reach: From Science to Policy", held in Vienna by the International Institute for Applied Systems Analysis (IIASA) from 24-26 October 2012. The conference theme was the development of integrated, multi-stakeholder and multi-national approaches to solve current and future global problems. More than eight hundred researchers, policymakers, and industry leaders participated. FEPL staff presented a poster on "The role of the laboratory in the farm-to-fork food safety chain".
- An oral presentation at the 2012 European Geophysical Union (EGU) Assembly in Vienna. The EGU meeting is one of the largest in Europe and featured a special session on Isotopes and Isoscapes as Tools for Forensic Provenancing. The FEPL presentation was on the effect of precipitation, geographical location and biosynthesis on New Zealand milk powder bulk and fatty acids D/H ratios. The utility of novel stable isotope measurements on the individual fatty acids from milk powders for determining the milk's origin was discussed. This work is an example of how the technologies being developed through the FEPL can be used to improve traceability systems for Member States.
- The FEPL also presented its work on food contaminant control in the final session, "Enhancing Food Safety" of the 2012 IAEA Scientific Forum, "Food for the Future - Meeting the Challenges with Nuclear Applications" held on 18 September 2012 during the 56th IAEA General Conference in Vienna, Austria.

In 2012, FEPL was also involved in planning the scientific programmes of international conferences through the Laboratory Head's inclusion in the scientific committees of:

- The EuroResidue VII Conference on Residues of Veterinary Drugs in Food, Egmond aan Zee, the Netherlands, 14-16 May 2012.
- The 2nd International Conference on Food Integrity and Traceability, Queen's University Belfast, UK, 8-10 April 2014.

CAPACITY BUILDING

The FEPL provided technical management for eighteen national and seven regional TCPs in 2012. The expertise available in FEPL and the methods and techniques developed were also used to support technology transfer to Member States through various train-the-trainers activities, both at Seibersdorf and in Member States. The FEPL hosted three individual fellowships and group training for six Scientific Visitors, totalling approximately eleven man-months. Two internships were also completed in the FEPL during this period, and one cost-free expert gained knowledge and experience whilst assisting in the implementation of FEPL activities.

Technology packages integrating bioassays and bio-monitoring screening tests for food and environmental contamination with physico-chemical and isotopic analytical methods were successfully developed, transferred and applied in Argentina, Chile, Costa Rica, and Uruguay. The methodology provides feedback to food chain stakeholders enabling them to optimise the use of agrochemicals, improving both environmental sustainability and food safety. Eight other countries are currently testing or validating the technology.

Sustainability of capacity building activities to improve food safety and quality through nuclear technology and networking

The FEPL was successful in a bid for funding from the USA under the Peaceful Uses Initiative, the objective of which is to support the IAEA in facilitating greater access for Member States to the peaceful uses of nuclear technology. Two train-the-trainers workshops at Seibersdorf and three regional workshops in Member States were held in 2012, the first year of this 3-year project, with more than 90 developing country scientists being trained in various aspects of food safety control.

Three regional training workshops for scientists from Latin America and the Caribbean were held in 2012. A training workshop on "Liquid Chromatography coupled to mass spectrometry (LC-MS/MS) and control of contaminants in food" was held in Panama City, Panama, from 17-27 April 2012 at the "Laboratorios de Residuos Tóxicos en Carnes y Control de Residuos de Plaguicidas en Plantas y Productos Vegetales" of the Ministerio de Desarrollo Agropecuario de Panama (MIDA). The workshop was organized in collaboration with the Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA) and the MIDA and was attended by eighteen scientists from seven countries. A second workshop, jointly organized by the FEPL in collaboration with OIRSA and the Belize Agricultural Health Authority (BAHA), on "Food Safety: From Farm to Fork" was held in Belize City from 27-31 August 2012, with the participation of twenty four representatives from eleven countries in the region. A third regional workshop on "Integrated analytical approaches for food traceability and contaminant control" was held in Montevideo, Uruguay, from 5-9 November 2012 with the participation of fourteen representatives from eight countries in the region.

Participants in the FAO/IAEA Workshop on "Food Safety: from Farm to Fork"

Two inter-regional train-the-trainers workshops were held in the FEPL in 2012. A workshop on "Food Safety: From Farm to Fork" was held from 29 June to 2 July 2012. Twenty-one scientists from eleven countries attended the workshop, which was held immediately following the European Pesticide

Residues Workshop (EPRW), taking advantage of the attendance of many developing country scientists at that conference. The objective of the workshop was to provide advice and guidance to developing country scientists to assist in setting up contaminant/residue monitoring programmes for food, with a special focus on pesticides. Practical demonstrations in FEPL focused on advanced analytical instrumentation such as high resolution/accurate-mass mass spectrometry, multi-residue method validation and radiotracer techniques.

An inter-regional workshop on "Radiotracer Techniques for Food Contaminant Control" was held from 27 September to 5 October 2012. The workshop had fifteen participants, representing nine countries.

The RALACA laboratory network

Ms Gadimorone being trained on LC-MSMS

A sustainable, formal laboratory network for food safety and environmental sustainability, the Red Analitica de Latino America y El Caribe (RALACA), was initiated and established with FEPL assistance. Sixteen laboratories are involved initially, with expansion planned for 2013, coordinated by the IAEA Collaborating Centre in Costa Rica. The RALACA network will play an important role in the future transfer of technology and methodology developed in the FEPL to the Latin America/Caribbean region, and will also greatly enhance the regions ability to pre-empt or react to food safety issues that arise.

Fellowships, Scientific Visitors and Interns

Two young scientists completed internships in the FEPL during 2012. Mr Wolfgang Dieter Werner, from EARTH University, Costa Rica, completed his one-year internship with the FEPL in August. Wolfgang worked with FEPL staff on the development of bioassays and bio-monitoring techniques as indicators of the effectiveness of pesticide application regimes and good agricultural practices in ensuring food safety whilst maintaining environmental sustainability. The protocols for the methods produced were transferred to Member States through a number of regional and national TCPs. In January 2012, Mr Sorivan Chhem-Kieth, from Concordia University, Canada, completed his eighteenmonth internship during which he worked with the FEPL and the Joint FAO/IAEA Division's Soil and Water Management and Crop Nutrition Laboratory on analytical methods for contaminant control in food and stable isotope methods for food traceability and soil erosion studies.

Ms Yao Minna, from the College of Food Science, Fujian Agriculture and Forestry University, China, joined the FEPL in July 2012 for a six-month period as a cost-free expert. Ms Yao gained knowledge and experience in FEPL and contributed to the work mainly on bio-monitoring techniques as indicators of food and environmental contamination, especially pesticides toxicity testing. She also assisted in other FEPL activities, including the preparation and implementation of a train-the-trainers workshop on the use of radiotracer techniques in food contaminant control.

The FEPL hosted six scientific visitors for a period of two weeks under the regional TCP RLA/5/060, "Harmonizing and Validating Analytical Methods to Monitor the Risk of Chemical Residues and Contaminants in Foods to Human Health". Mr Oscar I. Guardado Aguilar and Mr Cipriano A. Lopez Lezama from Nicaragua, Ms Brenda I. Checa Orrego and Ms Maddala A. Serrano Cortez from Panama, and Ms Silvia C. De Colombo and Ms Laura G. Mereles from Paraguay received training on the use of radiotracers in pesticide residues

analysis, method optimisation, uncertainty estimation, the use of stable isotopes in isotope dilution assays, and pesticide adsorption measurements in soils. The training period included participation in the FAO/IAEA interregional train-the-trainers workshop on "Radiotracer techniques for food contaminant control".

Three TC fellowships were completed in the FEPL in 2012. Mr Khaled El-Hawari, from the Laboratory for the Analysis of Pesticides and Organic Pollutants, National Council for Scientific Research, Lebanese Atomic Energy Agency, completed his four-month fellowship training period in January, related to TCP LEB/5/014. Khaled worked on methodology for the traceability of food and feeds using stable isotope ratio analysis. Mr Zhu Jie, from the Institute for the Application of Atomic Energy, Chinese Academy of Agricultural Sciences, joined the FEPL in April 2012 for a three-month fellowship focusing on analytical methodology for pesticide residues analysis using modern, rapid sample preparation techniques and gas chromatography–mass spectrometry. Ms Kefilwe Precious Gadimorone, Botswana National Veterinary Laboratory, commenced a three-month fellowship under TCP BOT/5/006 in May 2012, working with FEPL staff on the development and validation of a liquid chromatography–tandem mass spectrometry method for the detection of a range of antibiotic residues in animal-derived foods.

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Cooper, K.M., Whelan, M., Kennedy, D.G., Trigueros, G., Cannavan, A., Boon, P.E., Wapperom, D., Danaher, M. (2012). Anthelmintic drug residues in beef: UPLC-MS/MS method validation, European retail beef survey and associated exposure and risk assessments. Food Additives and Contaminants, 29:746-60.

Samsanova, J.V., Cannavan, A., Elliott, C.T. (2012). Screening methods for the detection of chloramphenicol, thiamphenicol and florfenicol residues in foodstuffs. Critical Reviews in Analytical Chemistry, 42:50-78.

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Byron, D.H., Cannavan, A., Sasanya, J.J. (2012). Report on Activities of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture Relevant to Codex Work (CX/RVDF 12/20/3 Add.1). Twentieth Session of the Codex Committee on Veterinary Drugs in Foods, San Juan, Puerto Rico, 7-11 May 2012.

Byron, D.H., Cannavan, A. (2012). Report on Activities of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture Relevant to Codex Work (CX/PR 12/44/4). Fourty-fourth Session of the Codex Committee on Pesticide Residues, Shanghai, China, 23-28 April 2012.

Byron, D.H., Cannavan, A. (2012). Report on Activities of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture Relevant to Codex Work (CX/CF 12/6/6). Sixth Session of the Codex Committee on Contaminants in Foods, Maastricht, the Netherlands, 26-30 March 2012.

EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
Laboratorios Microbioticos s/c/ Ltda, São Paulo, Brazil	Method development for food contaminants; technology transfer to Latin America
Centro de Contaminacion Ambiental (CICA), University of Costa Rica (UCR), Costa Rica	IAEA Collaborating Centre for eLearning and Accelerated Capacity Building for Food and Environmental Protection (EACB)
Institut für Lebensmittel-, Arzneimittel- und Umwelt-Analytik (ILAU), Germany	Collaborations on research activities linked to CRP D5.20.35 on "Integrated analytical approaches to assess indicators of the effectiveness of pesticide management practices at a catchment scale"
Division of Land and Water, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia	
Environmental Chemistry, Ecotoxicology, Pesticides and Radioactivity Department, State General Laboratory, Ministry of Health, Cyprus	
Austrian Agency for Health and Food Safety (AGES), Austria	Collaboration on accelerated capacity building for risk analysis and contaminants in food
Austrian Institute of Technology, Austria	Collaboration on nuclear techniques for research into interactions between environmental/food contamination 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 and Land Use, Queen's University Belfast, UK	Research and method development activities for food contaminants and food traceability
ASSET Centre, Queen's University Belfast, UK	Research activities in isotope-ratio methods for food traceability
Chemistry and the Environment Division, International Union of Pure and Applied Chemistry (IUPAC)	Collaboration on compendium of agrochemicals information

Institution	Topic
Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA)	Training for Member State scientists and regulators on food safety and quality
Agrolab, México	
Laboratorio Nacional de Insumos Agrícolas, Colombia	
Agilent Technologies, PA, USA	Training for Member State scientists in analytical techniques
RIKILT Institute for Food Safety, the Netherlands	Research into causes of food contamination with veterinary drug residues
Institute for Application of Atomic Energy, Department of Agro-Ecological Environment, Chinese Academy of Agricultural Sciences (CAAS), 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 Organization for Animal Health (OIE)	
World Food Programme	Control of mycotoxins in food stocks
Department for Applied and Engineering Chemistry, Faculty of Technology, University of Novi Sad, Novi Sad, Serbia	Transfer of natural plant toxins through the environment to food
International Federation for Animal Health (IFAH)	Quality control of trypanocidal drugs in sub-Saharan Africa
GALVmed	
UNODC	
University of Strathclyde, UK	
Manchester Metropolitan University, UK	
Laboratoire de Contrôle des Médicaments Vétérinaires, Dakar, Senegal	
Tanzania Food and Drug Authority, Tanzania	

Institution	Topic
University of Otago, New Zealand	Collaboration on the use of stable isotope measurements for traceability of foods Development and validation of new certified reference materials for stable isotope analysis Research into new stable isotope techniques for verifying the integrity of honey products
Food and Environmental Research Authority, UK	Collaborations on research activities linked to CRP D5.20.37 on "Implementation of Nuclear Techniques to Improve Food Traceability"

THE INSECT PEST CONTROL LABORATORY

EXECUTIVE SUMMARY

The research and development part of our work continued to focus on three insect groups (tsetse flies, fruit flies and mosquitoes), although in 2012, some work was carried out on Lepidoptera (moths).

In support of the tsetse eradication campaign in Senegal, the Insect Pest Control Laboratory (IPCL) carried out an introgression programme between female *Glossina palpalis gambiensis* flies originating from a Burkina Faso strain that had been adapted to mass-rearing conditions for several decades and males from a newly established strain from Senegal. This was to respond to the poor performance of the Burkina Faso strain when released in some parts of the target area in Senegal and to improve the robustness of the released strain. Mating studies in field cages indicated that the performance of the strain improved with each generation.

Work continued on the development of strategies to manage the virus that infects colonies of *Glossina pallidipes*, hampering colony production and growth. The use of a clean feeding strategy (each fly receives always fresh clean blood) or the mixing of blood with the antiviral drug Valacyclovir reduced or eliminated the prevalence of salivary gland hypertrophy (SGH) in the colony flies. Combining the two treatments reduced the time to eliminate SGH in the colony from 11 months in clean feeding alone to six months when clean feeding was combined with the drug treatment.

Work was initiated to assess the impact of antibiotic therapies on the microflora of the tsetse fly. The most important finding was that antibiotic treatment negated the expression of SGH symptoms in the F_1 progeny of virus-injected parents. It is likely that the removal of the symbiome might suppress the trans-generational transfer of the virus via the milk glands to the F_1 generation.

In the fruit fly rearing and quality control group, work continued to improve the mass-rearing of the olive fly, *Bactrocera oleae*, one of the most devastating pests of olives. In addition, four new wild strains (Croatia, France, Italy and Spain) of the olive fly were successfully colonized and field cage studies indicated complete mating compatibility between the various strains. A laboratory adapted strain competed successfully in field cages with their wild male counterparts, regardless of their origin.

In support of a CRP on "Resolution of cryptic species complexes of Tephritid pests to overcome constraints to SIT application and international trade" pre- and post-zygotic mating studies were continued with members of the *Anastrepha fraterculus* complex. Adults of a Colombian population exhibited strong temporal isolation with respect to the other tested populations (Mexico, Peru, Brazil, Argentina). Post-zygotic tests were also initiated between four members of the *Bactrocera dorsalis* complex (*B. dorsalis*, *B. papayae*, *B phillipinensis*, and *B. carambolae*).

As part of a collaborative agreement between the Joint FAO/IAEA Division and USDA/APHIS on post-harvest treatment of invasive fruit flies, work continued on hot water treatment, cold treatment and methyl bromide fumigation. Artificial fruits were developed to standardise the testing procedures and dose mortality curves using methyl bromide as a treatment were developed for eight fruit fly species at 15.5°C.

The fruit fly genetics group still maintains over 220 strains of fruit flies that carry mutations or translocations. Genotyping has started of all mass-reared genetic sexing strains of the Mediterranean fruit fly in all mass-rearing facilities all over the world.

In mosquitoes, it is only the female sex that regularly requires a blood meal; female mosquitoes are therefore solely responsible for the transmission of diseases. As a result, area-wide programmes that plan to include an SIT component can only release the male sex. Some years ago, staff at the IPCL developed a genetic sexing strain of *Anopheles arabiensis* (a vector of malaria) that was based on a dieldrin resistant mutation. Treating eggs or larvae of this *An. arabiensis* strain with dieldrin kills all the females while the male mosquitoes are resistant. Tests have now shown that male adults, treated as eggs for 2 hours with a 2 ppm dieldrin solution, contained small levels of dieldrin residues. Feeding male adults treated as eggs to gold fish showed bioaccumulation of the dieldrin in the fish. Work was therefore started to develop alternative ways of automatically separating females from males. Spiking the blood with ivermectin showed to have great promise and is being further pursued.

Field research in the project site in Sudan using laboratory reared sterile male *An. arabiensis* showed that males were able to survive for at least 5 days in the field and, were able to participate in mating swarms each night.

In terms of capacity building, the IPCL hosted nine cost-free experts, five consultants, five interns, two PhD students and nine fellows (the latter funded by the Department of Technical Cooperation).

In terms of services, the IPCL supplied numerous biological materials to various institutes all over the world, including 58,000 pupae of tsetse to 13 research institutes in Ethiopia, Germany, Italy South Africa, Switzerland, the UK and the USA, 30 shipments of various fruit fly species to ten institutions in France, Greece, Israel, Italy, Spain, and Tunisia, 60 shipments of dead fruit fly samples to 20 institutions in Argentina, Australia, France, Greece, China, Israel, Italy, Myanmar, Pakistan, Peru, South Africa, Spain, Turkey, and, the UK and 15 shipments of *An. arabiensis* eggs to Belgium Germany, South Africa, Sudan, Sweden, and the UK. Larval diet was sent to colleagues in Benin, Burkina Faso, Italy, Mauritius, Reunion Island, South Africa and Sri Lanka.

STAFF

Name	Title
Vreysen, Marc	Laboratory Head
Abd Alla, Adly	Virologist (Tsetse Flies)
Franz, Gerald	Geneticist (Plant Pests)
Bourtzis, Kostas	Geneticist
Gilles, Jeremie	Entomologist (Mosquitoes)
Caceres, Carlos	Entomologist (Plant Pests)
Parker, Andrew	Entomologist (Tsetse Flies)
Yamada, Hanano	Junior Professional Officer (Mosquitoes)
Targovska, Asya	Senior Laboratory Technician
Wornoayporn, Viwat	Senior Laboratory Technician
Adun, Henry	Laboratory Technician
Ahmad, Sohel	Laboratory Technician
Marin, Carmen	Laboratory Technician
Mohammed, Hasim	Laboratory Technician
Schorn, Elisabeth	Laboratory Technician
Soliban, Sharon	Laboratory Technician
Dammalage, Thilakasiri	Laboratory Attendant
Ibanschitz, Ottilie	Laboratory Attendant
Lapiz, Edgardo	Laboratory Attendant
Gembinsky, Keke	Laboratory Attendant
Sto. Tomas, Ulysses	Laboratory Attendant
Cancio, Elena	Laboratory Attendant
Wimberger, Tamara	Team Assistant

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

Tsetse flies

Colonies

The two main colonies kept at the Insect Pest Control Laboratory (IPCL) are *Glossina pallidipes* and *Glossina palpalis gambiensis*. Smaller colonies of *Glossina morsitans centralis* (n = 750), *Glossina morsitans morsitans* (n = 450), *Glossina brevipalpis* red eye (n = 830), *Glossina swynnertoni* (n = 16), *Glossina fuscipes fuscipes* (n = 153), and *Glossina pallidipes* Arba Minch (n = 45) were likewise maintained.

The main colony of *G. p. gambiensis* originated from the Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES), Burkina Faso. It was proposed to use this Burkina Faso (BKF) colony for an SIT operation in western Senegal, and initial trial releases using sterile male flies from this colony in the main target area were very promising. However, the flies had very high mortality when released in the Parc de Hann in Dakar. The IPCL was therefore asked to outcross the BKF colony with wild flies that were derived from pupae (SEN flies) from wild collected females from Pout, near Dakar. The outcrossing was performed by taking colony adapted virgin females from the BKF strain for mating with surplus males from the SEN strain. The generations were kept separate for four generations and then pooled to form the out-cross colony. This colony was transferred to the Slovak Academy of Sciences (SAS) in Bratislava, Slovakia in mid-2012 for further colony development. In each generation, mating competitiveness tests were carried out in standard field cages where introgressed males were competing against BKF males for SEN females. The introgressed strain progressively improved in mating participation with each generation. Once the introgressed colony at the SAS will have reached a suitable size, shipments of sterile male pupae from the introgressed strain will be initiated to Senegal to assess its field performance.

Clean feeding strategy for the Glossina pallidipes *colony*

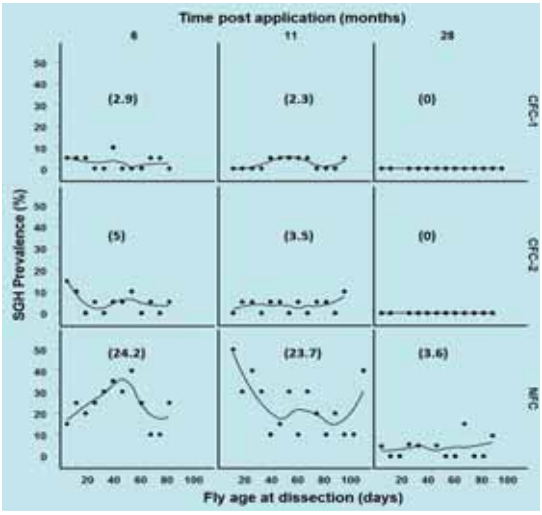

FIG. 1. Effect of long-term clean feeding system on SGH prevalence in G. pallidipes *colonies by dissection.*

As reported in previous activity reports *Glossina pallidipes* flies carry a virus that, in a certain proportion of individuals, leads to salivary gland hypertrophy (SGH) and these individuals also show reproductive abnormalities leading to reduced fecundity. In natural populations the prevalence of the virus was low (0.5-5%), but was higher (4-10%) in a colony that is maintained at the IPCL and that originated from Uganda and was very high (up to 77%) in a colony that is maintained in the Kality facility in Ethiopia. PCR analysis has confirmed that the virus is widely distributed in laboratory colony flies of other tsetse species.

Since mid-2009 management of the *G. pallidipes* colony was changed and a "clean feeding" strategy applied, i.e. the flies were always fed on blood that had not been used to feed any other flies to ensure that there was no salivary gland hypertrophy virus (SGHV) in the blood. Two clean feeding colonies (CFC1 and CFC2) were established whereby the second received blood used for the first clean feeding colony. The remainder of the colony (NFC) was fed with blood used for the two clean feeding colonies. The implementation of the clean feeding strategy in *G. pallidipes* colonies resulted in a complete elimination of SGH symptoms in the two clean feeding colonies and a significant reduction of the SGH prevalence in the normal feeding colony (Fig. 1). These results encouraged the transfer of this strategy to Member States and it was recommended to the colony manager of the tsetse mass-rearing facility in Kality, Ethiopia.

Use of antiviral drugs

We investigated previously the impact of two antiviral drugs (Acyclovir and Valacyclovir) on re plication of the SGH virus. Long term treatment of the blood meals with Valacyclovir was not toxic to the flies as indicated by normal pupae production. Feeding experimental flies for 48 months with blood contaminated with the virus but treated with Valacyclovir resulted, in comparison to untreated flies, in a significant reduction in SGH in dissected flies and a much reduced virus load. Due to these excellent results the treatment of blood with Valacyclovir to reduce the virus infection in *G. pallidipes* colonies was recommended to Member States and is being implemented in the tsetse rearing facility in Bratislava (Slovakia) and Kality (Ethiopia). Over the past four years we have continued this feeding regimen to monitor the possible development of antiviral drug resistance, but to date no such evidence has been detected.

Effect of combination of Valacylovir and clean feeding on reducing SGH prevalence of Glossina pallidipes *colony with high SGH prevalence*

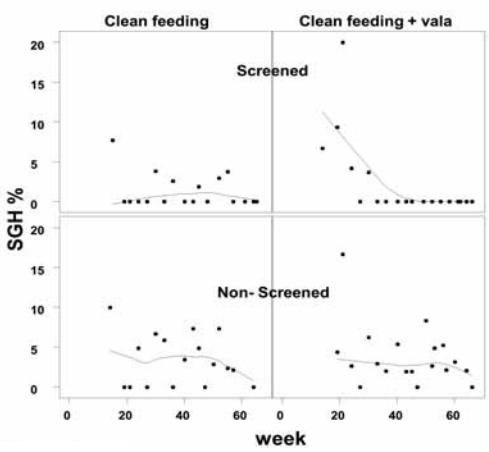

FIG. 2. Effect of the combination of clean feeding, screening flies with non-destructive PCR for virus infection and valacyclovir treatment on the expression of SGH syndrome.

To evaluate the time needed to reduce the SGH prevalence in a *G. pallidipes* colony with high SGH prevalence, flies from a contaminated colony with an average of 24% SGH were fed either on (i) clean blood supplemented with 300 µg Valacyclovir/ml or on (ii) contaminated blood supplemented with 300 µg Valacyclovir/ml. The dissection results indicated the absence of SGH symptoms in the clean feeding treatment with Valacyclovir while in the contaminated feeding 5% of flies still showed SGH. This result clearly indicates that the combination of clean feeding with Valacyclovir treatment reduced the time needed to eliminate SGH from a treated colony from 11 months in clean feeding alone to six months when Valacyclovir is added to the blood (Fig. 2).

Impact of antibiotic therapies on the tsetse fly microflora

Tsetse fly species are known to harbour a complex of symbiotic bacteria that are critical to their nutritional and reproductive fitness. In addition to the bacterial symbionts, many established tsetse colonies also harbour hytrosaviruses that are maintained at asymptomatic levels. Under certain circumstances, the asymptomatic state changes to a symptomatic condition, resulting in salivary gland hypertrophy that affects various host fitness parameters.

Bacterial symbionts play a critical role in maintaining the "immune status" in insects and in the case of tsetse flies, disruption of the symbionts led to increased host susceptibility to the important trypanosome complex. One may therefore speculate that alterations in *Wolbachia*, *Sodalis*, and/or *Wigglesworthia* levels may also be responsible for the switch from asymptomatic SGHV to the symptomatic SGHV state.

We set up a series of bioassays that examined the impact of the antibiotic ampicillin on both asymptomatic flies and flies injected with virus-infected gland extracts. Our hypothesis was that the antibiotic-induced immune suppression would stimulate increased virus titre in flies and potentially trigger the expression of SGH symptoms. Initially, male *G. pallidipes* exhibiting SGH symptoms were selected from the colony and used as a source to prepare virus inoculum.

The results indicate that intra-haemocoelic inoculation of *G. pallidipes* with virus preparation did not cause any initial perturbations in adult activity or fitness. Virus-challenged adults mated, imbibed blood meals and produced numbers of male and female F_1 progeny during the initial five weeks that mirrored those produced by control females. Surprisingly, intra-haemocoelic injection of the SGHV into adults did not increase the incidence of SGH in those flies; we expected that bypassing both the cuticle and gut barriers would have induced heavy infections and high incidence of SGH in virus-injected adults.

Impact of SGHV inoculation on F_1 progeny

In replicated assays, the frequency of SGH symptoms in the F_1 progeny produced at different larviposition cycles was determined. In control assays (N = 271) only two F_1 adults (0.7%) displayed detectable SGH symptoms whereas 59% of the F_1 adults produced by virus-injected females exhibited SGH symptoms. The prevalence of SGH in these newly emerged F_1 adults was associated with their larviposition cycle, and SGH symptoms increased from 4.5% in adults from the first larviposition cycle to 100% in adults from the fourth larviposition cycle. The ability of SGHV-injected females to induce high levels of SGH symptoms in the F_1 adults correlated with increases in viral titre in the parental generation; the F_1 adults from the third larviposition cycle were produced by mothers at 28-42 days post-injection that contained approximately 109 viral copies/fly. Therefore, we conclude that virus injection induced virus titres in adults that resulted in sufficient trans-generational transfer of virus to induce SGH symptoms during F_1 adult development.

The presence of Sodalis *and* Wigglesworthia *and the absence of* Wolbachia *in* G. pallidipes

The *G. pallidipes* laboratory colony used in our studies, like other tsetse flies, was expected to harbour species of both *Sodalis* and *Wigglesworthia* and possibly *Wolbachia*. Initial

comparisons among samples from control and virus-injected *G. pallidipes*, using both conventional PCR and qPCR, demonstrated that laboratory colony flies retained an average titre of 1-5 × 10^5 copies/fly of *Sodalis* and *Wigglesworthia* throughout the adult lifespan. Superinfection with the SGHV had no detectable impact on the symbiont titre in parent flies; in both treatments bacterial levels averaged 5 × 10^3 cells/fly at seven days and increased to 1 × 10^5 cells per fly by 21 days post-injection. The newly emerged F_1 male and female progeny sampled from the different larviposition cycles from both control and virus-injected treatments contained approximately 5- to 10-fold fewer copies of these symbionts than detected in parents.

Impact of ampicillin on Sodalis *and* Wigglesworthia

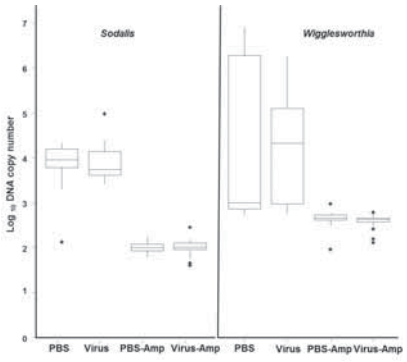

FIG. 3. The impact of ampicillin and PBS treatments on Sodalis *and* Wigglesworthia *titres in the F_1 generation of* G. pallidipes.

Groups of newly emerged *G. pallidipes* injected with either phosphate buffered saline (PBS) or virus gland suspension were fed blood three times per week supplemented with 40 µg ampicillin/ml of blood throughout adulthood. Ampicillin treatments caused a significant ten-fold reduction in *Sodalis* but did not totally clear *Sodalis* and the reduced levels observed after six blood meals remained constant throughout the parental sampling period. Unlike *Sodalis*, the titre of *Wigglesworthia* in flies injected with either PBS or the virus gland suspension were not impacted by being fed ampicillin-supplemented blood but increased with fly age from about 5 × 10^3 to 1 × 10^5 copy numbers per fly.

The ampicillin-induced reduction of *Sodalis* in the parent flies also impacted the bacterial levels in the F_1 progeny, male and female progeny from ampicillin-treated virus-injected adults lacking detectable *Sodalis* amplicons, regardless of the larviposition cycle, in conventional PCR reactions. Similarly, ampicillin treatment, although not suppressing *Wigglesworthia* titres in the parent generation, suppressed titres in the F_1 progeny (Fig. 3). qPCR demonstrated that genomic DNA from the F_1 of ampicillin-treated parents contained approximately 10^2 *Sodalis* and 2.7 × 10^2 *Wigglesworthia* copies/fly, significantly lower than those found in the progeny of non-antibiotic treated adults. Potentially, the antibiotic treatment cleared extracellular *Sodalis* and *Wigglesworthia* without impacting intracellular *Sodalis* infecting stem cells. It should be noted that the copy numbers detected in the F_1 progeny of ampicillin treatments are close to the detection limit of the qPCR, suggesting that the F_1 progeny are likely void of *Sodalis* and *Wigglesworthia*.

Impact of ampicillin treatments on Glossina pallidipes *and SGHV*

The ampicillin therapy, although dramatically lowering the bacterial symbiont levels of offspring, did not cause drastic impacts on the survival, reproductive behaviour, or fertility of treated *G. pallidipes* adults. Flies fed ampicillin supplemented blood readily mated and produced a normal complement of F1 offspring. Antibiotic treatment caused a minor increase in pupal mortality in the F1 from both the PBS controls (5%) and the virus-injected adults (8%). However, these impacts were much less than the 20% reduction in emergence observed

in an earlier study with ampicillin treatments of *G. morsitans morsitans*. Importantly, compared to the SGHV levels in adults fed control blood, the ampicillin treatment did not significantly change SGHV titres in either the PBS- or virus-injected adults.

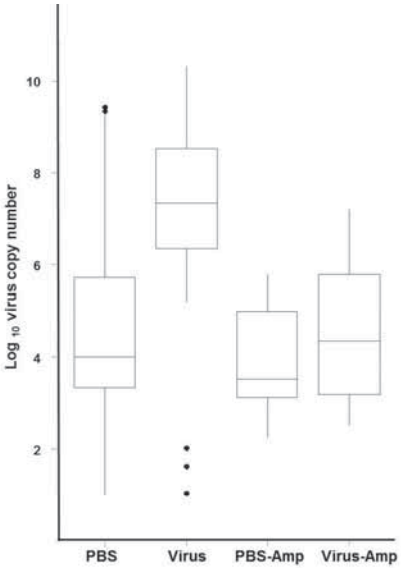

FIG. 4. Ampicillin treatment reduced the virus load in F_1 progeny of superinfected parents. Virus copy numbers detected by qPCR on genomic DNA extracted from the F1 progeny (N= 8) of superinfected parents (Virus) were significantly greater than levels detected in the F_1 progeny of asymptomatic control (PBS) flies. Feeding the superinfected parents ampicillin-supplemented blood meals (Virus-Amp) reduced virus copy numbers to levels similar to those detected in the asymptomatic PBS (control) and PBS-Amp flies. Black dots denote outliers.

Most notable is our finding that antibiotic treatment negated the expression of SGH symptoms in the F_1 progeny of the virus-injected parents. Virus titres in the ampicillin-treated, virus-injected parents were comparable to those of virus-injected adults fed untreated blood. However, the virus titres (5×10^4 copies/fly) of the F_1 progeny from the antibiotic treated virus-injected parents were similar to the virus titres (1×10^4 copies/fly) detected in the F_1 progeny from the PBS-injected controls and significantly less than the virus titres (~4×10^7 copies/fly) in the F_1 progeny from virus-injected parents fed unsupplemented blood. Therefore, removal of the symbiome might suppress the trans-generational transfer of the virus via the milk glands to the F_1 (Fig. 4).

The relation between Wolbachia *titre and sterility in inter-species hybrids of tsetse flies*

It is well known that some species of tsetse cannot hybridize with other species or can hybridize but produce only sterile offspring. The role of *Wolbachia* in inducing cytoplasmic incompatibility (CI) that leads to embryonic lethality is thought in many insect species to be the reason for this sterility of the F_1 in interspecies hybrids. To explore the impact of *Wolbachia* in interspecies hybrid sterility in tsetse, we started a series of interspecies crosses using *Glossina morsitans morsitans* and *Glossina morsitans centralis* treated with tetracycline (20 µg/ml). The primary obligate symbiont *Wigglesworthia* provides B vitamins to the tsetse, supporting reproduction in the females. As tetracycline drastically reduces or eliminates *Wigglesworthia*, the blood treated with tetracycline was also supplemented with yeast extract to (partially) rescue fertility. The results indicate that untreated female *G. morsitans centralis* mated with tetracycline treated male *G. morsitans morsitans* produced a high number of pupae, higher that *G. morsitans centralis* treated females mated with untreated *G. morsitans morsitans* males or when both sexes were treated with tetracycline. A possible explanation is that the tetracycline removes the *Wolbachia* from *G. morsitans morsitans* males, which could otherwise induce CI when mated with *G. morsitans centralis.*

Fruit fly rearing and quality control

Pre- and post-zygotic mating compatibility studies of olive fly strains originating from different geographical areas

The olive fly *Bactrocera oleae* is one of the most important pests of olives and the IPCL has been making serious efforts to improve the rearing of this pest. An important component of the SIT is to ensure mating compatibility of the released strain with that of the target population. Assessing mating compatibility of strains of different geographic origin will provide answers whether a strain that has been successfully colonized and mass-produced in a mass-rearing facility can be used for releases against populations of other geographical regions. In 2012 four new colonies were established at the IPCL: from Croatia (Kastel Stari, near Split), Italy (Ospedaletti, near Imperia) France (Nezigan L'ereque) and Spain (Valencia). Mating compatibility studies in field cages were carried out between all possible combinations of the four newly established strains before the 5th generation. In addition mating competitiveness studies in field cages were undertaken using a laboratory adapted hybrid strain of olive fly that originated from an old laboratory population (Democritus strain) backcrossed with wild material (males) collected from a distant geographical area (Israel).

FIG. 5. Bactrocera olea.

The mating studies indicated high mating compatibility among the olive fly populations from the four Mediterranean countries, with effective sperm transfer and good egg hatch in all cases. The mating latency (time between introduction of the flies and copulation) and copulation duration were similar in all combinations tested. Irradiated (100 Gy) male olive fly from the laboratory adapted hybrid strain successfully competed with their "wild" male counterparts regardless of their geographical origin. The fly strains from Italy and France undergoing colonization showed a stable mating propensity (e.g. no evidence of assortative mating) over a period of three generations.

Resolution of cryptic species complexes of Tephritid pests to overcome constraints to SIT application and international trade

Anastrepha fraterculus *complex*

As mentioned above, mating compatibility between populations from different geographical origins are a very important aspect for the development and implementation of the SIT. The South America fruit fly, *Anastrepha fraterculus*, is a wide ranging pest of fruits, i.e. from Argentina to Mexico. Earlier work already indicated mating incompatibility between certain populations giving indications that the species is actually a complex of cryptic species with different mating behaviour. In 2011 (see annual report 2011), researchers from Argentina furthered this research with more populations and the results indicated sexual isolation between populations from Peru and Brazil, and between populations from Brazil and Argentina. In 2012, the IPCL hosted seven visiting scientists from Argentina, Brazil, Colombia, Czech Republic, Mexico, and Peru to continue with experiments to test the mating compatibility

of *A. fraterculus* populations belonging to five different morphotypes: *A. spp. Mexican aff fraterculus* (Xalapa, Mexico), *A. spp. 4 aff fraterculus* (Peruvian morphotype, Piura Peru), presumably *A. sp. 2 aff fraterculus* (Parnarimi, Bahia, Brazil), *A. spp. highland areas from the Andean region aff fraterculus* (Ibague, Colombia), and *A. sp. 1 aff fraterculus* (Tucuman, Argentina).

Adults from the Colombian population exhibited strong temporal isolation with respect to all tested populations. Mating activity of individuals of this morphotype began around 4 pm with a peak around 6 pm, while adults of the Argentinean and Mexican populations began mating at release around 8 am and had finished sexual activity by 11 am. Adults of the Peruvian morphotype mated around noon and could extend sexual activity into early afternoon providing the opportunity for some temporal overlap that resulted in some heterotypic crosses. Sexual isolation appeared to be strong between these morphotypes.

Pheromone samples and cuticle hydrocarbons extraction of these populations have been collected for further analyses in collaboration with researchers in Argentina, the Czech Republic and USA. Proteins from the male accessory glands were extracted and characterized for several populations and morphotypes and are showing in some cases distinct banding patterns.

Post-zygotic isolation among adults of the Colombian population and adults of the other morphotypes was tested. Different levels of post-zygotic isolation were detected. In all cases there was a 50-70% reduction in fertility for heterotypic crosses when compared to the homotypic parental crosses. Fertility of F_1 hybrids appeared nevertheless to be somewhat restored. Collective evidence seems to indicate that the Colombian morphotype is a distinct biological entity.

Bactrocera dorsalis *complex*

The IPCL also continued working towards resolving species limits among morphologically cryptic pest taxa of the *Bactrocera dorsalis* complex. The IPCL hosted two visiting scientists from Australia and China to support these activities. The focus of this research was to carry out pre- and post-zygotic compatibility studies to 1) complete post-zygotic compatibility tests among populations of *B. dorsalis* (Thailand), *B. papayae* (*Malaysia*), *B. philippinensis* (Philippines) and *B. carambolae* (Suriname); and 2) assess sexual compatibility between *B. dorsalis* and *B. invadens* to help resolve their on-going taxonomic uncertainty. Initial field cage tests between *B. dorsalis* (Wuhan, China) and *B. invadens* (Kenya) demonstrated random mating (using the Index of Sexual Isolation) with no evidence of post-zygotic incompatibility between these two species (e.g. egg hatch, larval survival, pupal survival, and sex ratios); future tests among flies from India, Myanmar, Pakistan and Sri Lanka are anticipated. Much of this work will be completed in 2013. Furthermore, pheromones will be collected of the different populations and specimens will be preserved for additional analysis (e.g. wing geometric morphometrics) that will also contribute towards resolving their biological relationships.

Progress with post-harvest treatments of invasive fruit flies

The IPCL has been collaborating for several years with the USDA-APHIS Center for Plant Health Science and Technology (CPHST) on a joint project entitled "Development of phytosanitary and regulatory treatments for exotic tephritid fruit flies". The rationale for this project is that many countries cannot export their horticultural products due to the presence of a number of tephritid fruit fly pests that also pose a high-level threat of entry into the USA and other countries; furthermore, approved quarantine treatments are lacking for several important species. Adequate post-harvest treatment schedules for these pests are therefore required. Once these treatment schedules have been developed they can be used by potentially exporting countries in combination with pre-harvest pest suppression measures, in order to facilitate the export of their produce by minimizing or preferably eliminating the risk of introducing these exotic pests into importing countries. In addition, frequent outbreaks in the USA and bordering countries have resulted in temporary domestic quarantines that require regulatory treatments to be available for growers in outbreak areas prior to moving fruit and vegetables to both domestic and international markets.

In 2012, research was conducted on three post-harvest treatment types: hot water immersion, methyl bromide fumigation and cold treatment. Additionally, the collection of data on developmental and biological characteristics of fruit fly species of interest is on-going. The IPCL is currently rearing 14 species of invasive tephritid flies (with some 30 different strains) and this affords an excellent opportunity for comparative research that is not possible in any other facility in the world.

For the assessment of hot water treatment schedules, "artificial fruits" were developed to standardize the testing procedure so we can rapidly assess relative tolerance among the different tropical species that are available at the IPCL. Validation using mango as a host for three fruit flies species (*Bactrocera invadens*, *B. zonata*, and *Ceratits capitata*) is in progress and is scheduled for completion in 2013. This research will aid in the timely completion of a major goal of this cooperative project, which is the development of a hot water treatment schedule for *B. invadens* and *B. zonata* on mango, invasive pest species that have recently invaded the African continent. Preliminary work on mango has been completed, including the development of infestation techniques and experimental design.

FIG. 6. Fruit flies B. zonata *infesting fruits (upper),* B. zonata *(lower left),* Anastrepha ludens *(lower right).*

Similarly, a standardized testing procedure to assess Tephritid tolerances to cold treatment has been developed. Validation of this is scheduled to occur in 2013. A cold treatment quarantine schedule for *B. zonata* on oranges (1.67°C for 18 days) and a 17-day confirmatory test have been completed. This study led to

the development of a new treatment schedule for this pest species on citrus. Additionally, verification of the most tolerant developmental stage to cold treatment for *Anastrepha ludens* on grapefruit has been initiated, with planned completion in early spring 2013.

While methyl bromide has been scheduled to be phased out in many countries, it remains an important tool for the prevention of invasive species entering the USA. *In vitro* tolerance assessment of a range of species is a specific goal of this work plan. Dose-mortality curves that can be used as a guide for methyl bromide fumigation have been developed at 15.5°C (five treatment levels) *in vitro* for the third larval instar of eight fruit flies species. This study will be followed by another study using the same methods at 5°C that is scheduled to be completed in early 2013. Based on the results of these tests, we will be able to define the most tolerant life stages of different target species.

Work on *Anastrepha grandis* is on-going pursuant to the goals of this cooperative research. Much effort has been necessary to develop a laboratory colony suitable for testing. Successful infestation techniques for this tephritid in zucchini and pumpkin have been developed and data on egg/embryo and larva recovery is currently being analysed. Collection of biological information related to *A. grandis* development is on-going, the goal of which is the development of a protocol for artificial rearing of *A. grandis*. Collaboration is ongoing within ICPL to assess possible symbionts that could be beneficial in developing an artificial rearing diet. Once insects can be reared on artificial diet, we will then be able to resume our work on treatments for *A. grandis*.

Fruit flies genetics group

Fruit fly species and strains kept at the Genetics Group

The Genetics Group at the IPCL maintains more than 220 strains of four fruit fly species (*Ceratitis capitata*, *Bactrocera dorsalis*, *Bactrocera cucurbitae* and *Anastrepha ludens*) that are reared on a carrot powder-based larval diet. Most of these strains carry mutations or translocations and are unique in the world.

Ceratitis capitata *genotyping*

Work has started to genotype all mass-reared genetic sexing strains (GSS) of *Ceratitis capitata* that are used around the world. The goal is to develop an easy and robust method to identify mass-reared flies destined for release programmes and discriminate them from wild target populations (or invasive flies). We are using three approaches to characterize the GSSs: phenotypic, cytogenetic and molecular. The phenotypic analysis is based on the detection of three markers: *white pupae* (*wp*), *Sergeant*^{2} (*Sr*^{2}) and *temperature sensitive lethal* (*tsl*). Males should be *wp+* (pupae have brown colour) and *tsl+* (temperature tolerant) while females should be *wp* and *tsl*. The *tsl* mutation allows the separation of the two sexes as early as the embryonic stage. Variants of the VIENNA 8 strain (VIENNA 8-*Sr2*) also carry the dominant mutation *Sr*^{2} on the Y-autosome translocation, while the females of this strain are wild type with respect to this marker.

The cytogenetic analysis includes the detection of the chromosomal breakpoints associated with the translocation and/or inversion events that characterize the VIENNA 8 and VIENNA 7 GSS. The molecular analysis is based on four mitochondrial haplotypes [RFLP pattern] using four restriction enzymes: EcoRV, XbaI, MnlI and HaeIII. The AAAA haplotype is predominantly found in the Mediterranean region, but may also be occurring in Central America. If the mitochondrial DNA PCR-RFLP based analysis is not enough to discriminate between the various mass-reared GSS, then microsatellite markers will be considered too. The advantage of using RFLP is that it can be performed at almost any laboratory.

Genotyping tephritid species via mitochondrial genomics

Our goal is to develop (or improve existing) genotyping approaches of SIT targeted tephritid species using mitochondrial DNA markers. This can be done either via a PCR-RFLP or a DNA-sequencing approach. As a proof of principle, the mitochondrial genome of the following species / strains is being sequenced: *C. capitata* (Antigua GSS), *B. oleae* (Greek lab strain), *A. fraterculus* (Ica and Tucuman strains). Our primary objective is to genotype all mass-reared genetic sexing strains (GSS) that are used around the world. The mitochondrial genome of all of these strains is almost completed. Several potential single nucleotide polymorphisms (SNPs) were identified for *C. capitata* (Antigua) when compared with the available genome in the database (*C. capitata* Benakeion strain). It remains to be seen whether these sites will be useful for discriminating SIT flies from wild flies. Similar work and analysis is in progress for the *B. oleae* (Greek lab strain). In respect to *A. fraterculus*, this is the first attempt to provide the complete DNA sequence for the mitochondrial genome of this species.

Mosquitoes

Research in the Mosquito Group at the IPCL continued to focus on two main pests of human diseases, i.e. Anopheles arabiensis, a vector of malaria, and Aedes albopictus, a vector of dengue and Chikungunya.

Genetic Sexing for Anopheles arabiensis

Area-wide integrated pest management programmes against mosquitoes with an SIT component can only be implemented when the female mosquitoes are removed from the production line, especially in those target areas where the disease they transmit is endemic or recurrent. Female mosquitoes are the sole sex that requires regularly a blood meal, and hence they are the sole transmitters of diseases. Automatic sex separation systems are therefore required that need to ensure accurate and efficient separation of potentially large amounts of male and female mosquitoes. Male and female mosquitoes of species such as *Ae. albopictus* can be separated up to a certain degree using the sexual size dimorphism of the male and female pupae. Such an approach however does not work with a species like *An. arabiensis*. A few years ago, the IPCL developed a genetic sexing strain using classical genetic methods that was based on a dieldrin resistant mutation, i.e. treatment of larvae or eggs with dieldrin killed all female mosquitoes whereas males were resistant and could be used for potential releases. The strain has obvious disadvantages, i.e. its low productivity is hampering optimal production, treatment as eggs reduces the overall survival of adult males, the dieldrin treatment is a hazard for staff manipulating the insecticides, the use of insecticides in a large mass-rearing facility is not a recommended practice, etc.

Research was conducted to assess potential residues in adult male *An. arabiensis* after the treatment with dieldrin and an assessment was made to determine whether there is any evidence of bioaccumulation of the dieldrin in the food chain after feeding goldfish with adult mosquitoes that had been treated with dieldrin as eggs. Male *An. arabiensis* mosquitoes, treated as eggs for 2 h with a 2 ppm dieldrin solution, were found to have a mean dieldrin residue of 28.06 ± 2.87 ng/g. Analyses of the fish fed solely with dieldrin-treated male mosquitoes showed an accumulation of dieldrin ranging between 23.9 and 72.3 ng/g. Although the levels of dieldrin residues found in adult males were very low for individual mosquitos, releasing several million treated mosquitoes into the environment on a daily basis might result in a considerable overall environmental residue burden depending on the size of the treated area and the dispersion and dilution of the insects released. This is not acceptable in view that the experiments with the gold fish indicate that the insecticides residues in the mosquitoes can be transferred up the food chain upon ingestion by natural predators.

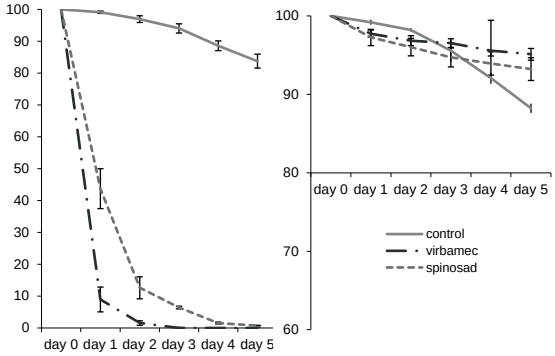

FIG. 7. Elimination of female Anopheles arabiensis *(left) and males (right) from the population over 5 days in control cages (solid line), in cages fed with spinosad-spiked blood (dotted line), and ivermectin-spiked blood (semi-dotted line).*

Alternative methods for the separation of the sexes of *An. arabiensis* were therefore investigated, such as the spiking of blood meals with toxicants to kill the females. Ivermectin was shown to give the best results, eliminating 99% of the females after 2 days without any adverse effects on male survival and mating ability (Fig. 7). As Ivermectin is an acceptable substance in terms of environmental and health safety, the blood spiking method is effective, simple, and has potential for application across many other mosquito species that currently lack alternative sexing methods. It requires further testing on a larger scale to assess its suitability for use in a large-scale mass-rearing operation.

Assessing competitiveness in mosquitoes

Adequate sexual competitiveness of sterile released insects is one of the most important requirements for successful SIT. To study the competitiveness in mosquitoes such as *An. arabiensis* presents special challenges as the mating occurs in swarms, at night and the copulation mostly lasts only seconds. Work was conducted to assess which type of cage could be used to evaluate competitive behaviour in *Anopheles* mosquitoes. Using three cage types of different dimensions, it was shown that insemination decreased with increasing dimensions of the cage. In the largest cage (200 × 200 × 200 cm) used (preferred cage as the larger the cage, the more natural the behaviour of the insects studied), about half of the females were still inseminated, which was deemed sufficient to assess mating frequencies of the different types of males involved. In a first test with adult males irradiated with 70 Gy as pupae, untreated males and virgin females at a 1:1:1 ratio, hatch rates of eggs were 69.3% compared to 80.6% for only untreated males and 18.2% for a cage containing only irradiated males. This clearly indicated that the competitiveness of the treated males was lower than that of the untreated males under those experimental conditions. This needs to be repeated to validate the results.

Swarm participation of Anopheles arabiensis: *observations in the field*

A study was carried out to investigate the capability of released sterile *An. arabiensis* males to survive, disperse and participate in swarms, and the effect of distance from the release site to the swarm position, using mark-release-recapture techniques. The *An. arabiensis* (Dongola strain, Sudan) used for the trial was cultured at the Tropical Medicine Research Institute in Soba (Khartoum, Sudan) and released at Merowe, Northern State, Sudan. Three groups of 300 male mosquitoes (treated as pupae with 70 Gy of gamma radiation) were released at a distance of 50, 100 and 200 m from the main swarm. Marked male mosquitoes were recaptured in the main swarm on five evenings following the releases, showing that some released males were able to survive at least five days in the field. In each of the releases, the proportion of released sterile males caught decreased with the distance from the release point. Of the 900 males released each day an average of 4.8±2.1, 2.6± 2.4 and 1.8±1.6 % were caught in the swarms at a distance of 50, 100 and 200m from the release point, respectively. This however does not mean that the majority of the released males were not available for population suppression, as they might be participating in swarms outside of the experimental area. Further studies will be carried out to assess their competitiveness in the swarm and their ability to successfully copulate and transfer sperm.

Development of mass-rearing equipment for Aedes albopictus

The further development of mass-rearing equipment was carried out in close collaboration with staff of the IAEA collaborating centre "Centro Agricoltura Ambiente G. Nicoli", located in Crevalcore, near Bologna, Italy. A new mass-production cage was developed that was self-contained with devices for blood feeding, pupal introduction, cleaning and egg collection. Effects of cage volume and adult density were investigated on egg production and survival. As a result, a cage of dimensions 100 ×10 × 100 cm was selected that could be stocked with up to 130 adults per litre of cage volume.

A larval tray for the mass-rearing of *An. arabiensis* was earlier developed at the IPCL[2]. Similar trays were used to assess its suitability for rearing *Ae. albopictus*. Various parameters were investigated, such as the effect of larval density, diet schedule, brewer's yeast, water temperature and the effect of stacking the trays in a rack on the development of the larvae. Larvae were kept at densities of 2, 3 and 4 larvae per ml of water. The addition of brewer's yeast to the diet improved the selectivity of the separation of the sexing that is based on pupa size, but fewer pupae were produced in comparison to a diet that had no brewer's yeast added. Keeping larvae at lower temperatures (25 and 26°C) improved pupal productivity but the number of male pupae that passed the sieve decreased, resulting in an unacceptable increase in the percentage of females passing through the sieves. Larval development when reared in isolated trays was similar to that of larvae reared in trays that were stacked inside a dedicated rack. The results obtained from rearing in the rack setting confirmed that the optimal water rearing temperature of 28°C produced a consistent level of productivity and male separation with mechanical sexing procedures.

[2] See activities report of the Entomology Unit 2009, p. 45.

CAPACITY BUILDING AND SERVICES

Capacity building

In 2012, the IPCL hosted nine cost-free experts (CFE), five consultants (C), five interns, two PhD students and nine fellows (the latter funded by the IAEA's Department of Technical Cooperation) in the following areas:

Name	Country	Status	Duration	Topic
Abraham, Solana	Argentina	CFE	1 mth	Mating compatibility studies with members of the *Bactrocera dorsalis* and *Anastrepha fraterculus* complexes to disentangle their taxonomic status and thereby remove constraints for SIT application and to facilitate international trade
Bo, Wang	China	CFE	12 mth	
Brizova, Radka	Czech Republic	CFE	2 wk	
Devescovi, Francisco	Argentina	CFE	1 mth	
Roriz, Kelly	Brazil	CFE	1 mth	
Rull, Juan	Mexico	C	1 mth	
Schutze, Mark	Australia	CFE	3 wk	
Rempoulakis, Polychronis	Greece	C	6 mth	Rearing of fruit flies
Fontenot, Emily	USA	CFE	12 mth	Post-harvest treatment of fruit flies
Myers, Scott	USA	CFE	1 wk	
Demirbas, Guler Uzel	Turkey	Intern	1.5 mth	Developing management strategies for the tsetse virus in support of the tsetse eradication project in Ethiopia and other East African countries
Drion, Boucias	USA	CFE	3 mth	
Kariithi, Henri	Kenya	PhD	10 mth	
Damiens, David	France	C	12 mth	Developing mass-rearing techniques and the SIT package for disease transmitting mosquitoes
Lees, Rosemary (partly paid by TC)	UK	C	6 mth	
Madakacherry, Odessa	USA	Intern	12 mth	
Maiga, Hamidou	Burkina Faso	Intern	6 mth	
Ndo, Cyrille	Cameroun	C	6 mth	
N'Do, Severin	Burkina Faso	Intern	6 mth	
Oliva, Clelia	France	PhD	6 mth	
Mohamed, Monib	Egypt	Intern	2 mth	Genetics of fruit flies
Castañeda, Rosario	Colombia	Fellow	1 mth	Fruit flies
Chakroun, S.	Tunisia	Fellow	3 mth	Moths
Essop, Leyya	South Africa	Fellow	2 mth	Mosquitoes
Fetawoke, E.A.	Ethiopia	Fellow	1 wk	Tsetse

Name	Country	Status	Duration	Topic
Julienne, S.A.	Seychelles	Fellow	1 mth	Mosquitoes
Manangwa, O.	Tanzania	Fellow	3 mth	Tsetse
Manilal, Y.Y.	South Africa	Fellow	2 mth	Mosquitoes
Niain'ny Felamboahangy, L.N.	Madagascar	Fellow	1 mth	Mosquitoes
Nolasco, Norma	Peru	Fellow	1 mth	Fruit flies

Services

In 2012, the IPCL supplied 58,000 pupae of tsetse (*Glossina pallidipes*, *G. brevipalpis*, *G. palpalis gambiensis*) to 13 research institutes in Ethiopia, Germany, Italy South Africa, Switzerland, the UK and the USA; 30 shipments of different fruit fly species to ten institutions in France, Greece, Israel, Italy, Spain, and Tunisia; and 60 shipments of preserved fly samples to 20 institutions in Argentina, Australia, France, Greece, China, Israel, Italy, Myanmar, Pakistan, Peru, South Africa, Spain, Turkey and UK. The IPCL also supplied 15 egg shipments of *An. arabiensis* to Belgium Germany, South Africa, Sudan, Sweden, and the UK. Larval diet was sent to colleagues in Benin, Burkina Faso, Italy, Mauritius, Reunion Island, South Africa and Sri Lanka.

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EXTRABUDGETARY SUPPORT

In 2012, the IPCL received extrabudgetary resources from the USA under:

- the USDA/APHIS agreement "Development of phytosanitary and regulatory treatments for exotic tephritid fruit flies" (Euro 144,589) and

- the Peaceful Uses Initiative (PUI) to support the project "Contributing to Agricultural Development in West Africa through the Control of Tsetse Flies and the Trypanosomosis Problem" (Euro 407,689).

THE PLANT BREEDING AND GENETICS LABORATORY

EXECUTIVE SUMMARY

Sustainable crop production is a fundamental requirement for food security. Yield is the number one concern of farmers and crops must be bred that have a high yield potential. Today crop yields are threatened by increased incidence of pests and diseases and by drought and salinity and other environmental factors. Many crops are grown on sub-optimal lands in desperate efforts to produce more food. Harvests in traditional growing areas have also become increasingly uncertain due to climate change. Constraints on crop yields are global phenomena and have global consequences; crop failure in one country not only affects local food security but affects the supply chain and increases commodity prices in world trade, making imports more expensive, a double blow for which developing countries are particularly vulnerable. Crops are challenged by biotic and abiotic stress at all stages of agricultural production systems, from sowing, germination, seedling establishment to flowering and harvest; susceptibility at any one stage can lead to crop failure. At the Plant Breeding and Genetics Laboratory we believe these challenges can be met through research and development in mutation breeding.

The Plant Breeding and Genetics Laboratory (PBGL) is an integral part of the Plant Breeding and Genetics Section and supports methods in plant mutation breeding in Member States for food security. These goals are reached through research and development activities in: 1) mutation induction; 2) screening for desired mutants, and 3) as of 2012, advancement of selected mutant lines. The technologies developed are transferred to Member States through training programmes, the dissemination of manuals, protocols and guidelines and the provision of mutation induction services. As such, all the work of the PBGL is driven by Member State demands and the PBGL works directly with the plant breeders from the Member States on specific traits for crop improvement. This involves a wide range of crop species, from both tropical and temperate regions.

Over 3200 improved mutant varieties have been developed in over 200 crop species around the world. The majority of these have been produced using gamma irradiation. However, gamma emitters involve radioactive isotopes and these are now increasingly subject to highly restrictive import and export regulations. X-ray irradiation is a sound and user-friendly alternative for plant mutation induction, and there is an urgent need to develop protocols and to provide training for plant breeders. In 2012 the PBGL produced the first protocol for X-ray mutagenesis of seed crops for dissemination to Member States.

The PBGL has continued its work in developing practical protocols applicable in the laboratories of most developing countries. For example, a basic technique in mutation screening is DNA extraction for which a low cost, non-hazardous method has been developed. Other protocols developed in 2012 include screening for salt tolerance in rice, wheat and barley.

In 2012, the PBGL finalized research into the genetic effects of mutagenesis and chimeras in shoot apical meristems of banana. This work was published in the peer reviewed Plant

Biotechnology Journal and highlighted on the cover of the journal. The research results show that chimera problems are resolved earlier than previously thought and this has practical significance in developing mutant lines in vegetatively propagated crops, such as banana, faster.

A new activity for the PBGL has been the initiation of accelerated methods for mutation breeding. The aim is to greatly reduce the time taken for mutant variety production. Mutation induction takes seconds/minutes, mutation detection (such as screening for salt tolerance) takes a few months, but the main time constraint in mutation breeding is the time taken to develop a selected mutant line into a variety for farmers to grow. For a relatively fast breeding annual crop, such as wheat and rice, traditional breeding methods take up to ten years. In today's rapidly changing world this is simply not fast enough. The needs of Member States are urgent and immediate and the speed of climate change demands increasingly rapid responses. Thus, the PBGL has initiated R&D activities in biotechnologies that accelerate mutant line development, such as rapid generation cycling, embryo culture and marker assisted selection.

Further highlights in 2012 include the initiation of a new national plant mutation breeding programme in the State of Palestine; the publication of a major reference book on "Plant Mutation Breeding and Biotechnology"; celebrating the 50th anniversary of the Seibersdorf Laboratories; and the appointment of a new staff member, Mr Abdelbagi Ghanim. In addition, new equipment for DNA sequencing was procured and funds were obtained for the refurbishments of our 30-year old glasshouse.

In the area of human capacity building, the PBGL trained 19 fellows from 13 countries and hosted six scientific visitors and two consultants.

Requests for irradiation services continued to increase in 2012; we received and responded to 37 requests for both gamma and X-ray irradiation from 21 Member States for 23 plant species.

STAFF

Name	Title
Forster, Brian Peter	Laboratory Head
Till, Bradley John	Plant Breeder/Geneticist
Ghanim, Abdelbagi Mukhtar Ali*	Plant Breeder
Matijevic, Mirta	Technician
Jankowicz-Cieslak, Joanna Beata	Technician
Berthold, Guenter	Field/Greenhouse Worker
Draganitsch, Andreas	Technician

Name	Title
Bado, Souleymane	Technician
Huynh, Owen Anthony	Technician
Seballos, Gilbert	Laboratory Attendant
Hofinger, Bernhard**	Technician
Lorenz, Anne	Implementation Assistant
Mletzko, Joanna Malgorzata	Team Assistant

* Joined the PGBL in August 2012.

** Joined the PBGL in August 2012 as a temporary replacement during the maternity leave of Joanna Jankowicz-Cieslak.

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The world faces severe global challenges in its efforts to achieve food security:

- The human population is currently growing at a rate of 1 billion people every 11-12 years.
- Arable land is restricted to some 13.8 million km^2 (9.3% of the earth's land area).
- Climate change is causing static or even declining global crop yields.

Can plant mutation breeding and genetics help meet such global challenges in food security?

There is no doubt that food security issues need to be met by an array of responses, including those provided by all FAO/IAEA Agriculture & Biotechnology Laboratories at Seibersdorf. However, the production of plant varieties with the genetic potential for high and stable yields is a basic, fundamental requirement. This is an on-going battle as the varieties of today will not serve for tomorrow. Climate change is already having a detrimental impact on crop yields; in Europe wheat yields have remained static over the past 10 years and in northwest Africa rice yields are in decline. Climate change brings increased risks of abiotic and biotic stresses and new more resilient crops are urgently needed that tolerate salinity, drought, flooding and storm damage; crops also need to be resistant to pests and diseases. Climate change is expected to hit developing countries in hot regions the hardest; it has been estimated that for every 1°C increase in temperature there will be a 1.7% drop in yield. The mandate of the PBGL is driven by the demands of Member States (MSs). It has three major components to carry out: 1) Research & Development, 2) Training and 3) Irradiation Services directed at mutation induction, mutation identification and accelerated breeding, i.e. the rapid development of promising mutants for plant breeding. At the PBGL we believe that these challenges can be met through science, the development of innovative technologies and knowledge transfer to MSs.

Mutation induction

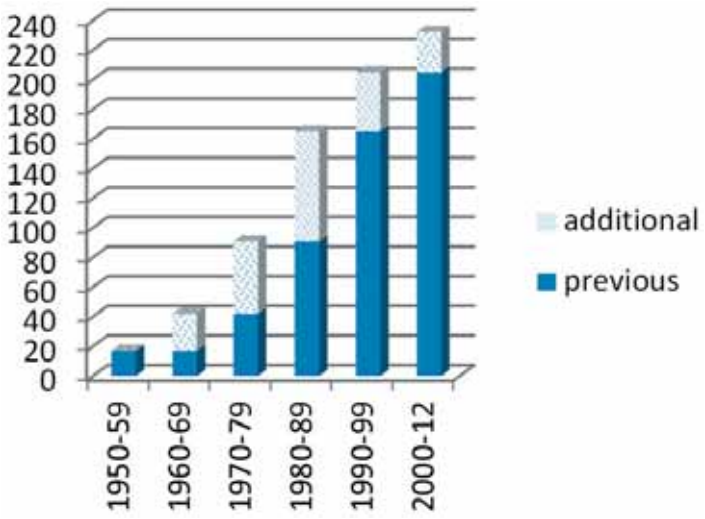

FIG. 1. Increasing numbers of plant species with induced mutant varieties over time; the number of new additional species for a given time period is illustrated as the top section of each column.

Mutation induction has been a very successful strategy in delivering new genetic variation to plant breeders; over 3,200 mutant crop varieties have been released worldwide. There is a continuing expansion of mutation varieties in more and more species (Fig. 1). Over 60% of these mutant varieties have been developed through gamma irradiation (*http://mgvs.iaea.org/*). However, increasing security restrictions imposed on the shipment of radioactive gamma emitters severely limit the use of gamma for plant mutation induction by Member States. In addition gamma emitters, because of radioactive decay, need to be refurbished every 10-15 years. Member States therefore need an alternative and at the PBGL we are developing the potential for X-ray induced mutagenesis. X-ray irradiation has advantages over gamma in that it does not involved radioactivity, the machines are user-friendly, widely available and can be switched off when not in use. In 2012 we developed our first protocol for X-ray mutagenesis of seed crops, which is available on our web-site: *http://www-naweb.iaea.org/nafa/pbg/index.html*

Mutation detection – genotyping

Mutation-assisted breeding consists of several major steps, including mutation induction, mutation screening, advancement of selected material, varietal testing, and varietal release. Up until 2012, the PBGL focused mainly on assisting Member States in the first two steps of this process: efficient implementation of mutation induction and screening. This work comes in the form of provision of technical services, development of protocols and guidelines and adaptive research and development activities.

The act of mutation screening can be broadly defined as the characterization and selection of mutant plants that possess interesting or useful variation. Mutation screening can be divided into two categories: genotyping and phenotyping. Each has its own unique advantages, and many breeding programmes, including those of large multi-national seed companies, make use of both approaches. In the area of genotypic screening, the PBGL is investing in new methodologies to recover mutations in genes of interest and in developing DNA diagnostic markers for efficient selection in plant breeding.

FIG. 2. The PBGL's studies on mutation induction and inheritance in banana were published in Plant Biotechnology Journal in 2012. The work was chosen to be highlighted on the December cover of the journal. The cover image shows the mutant banana population growing in the greenhouse at Seibersdorf (left), mutation discovery assays (top right) and DNA sequence validation of identified mutations (bottom right).

Given the success of induced mutation in plant breeding programmes over the last 70 years, the casual reader might be forgiven for thinking that the scientific community already knows everything about the effect of different mutagens and different mutagenic dosages on the DNA sequence of crop genomes. In fact, there are still major gaps in our understanding of the effects that mutagens have on plant genomes. To date, most of the work has focused on seed propagated crops. Less is known about vegetative crops that reproduce entirely or mostly *via* asexual methods. One issue with the application of induced mutations in vegetatively propagated tissues is that mutagenesis causes different mutations in the different cells of the treated tissue. The result is a genotypic mixture (heterogeneity) that is referred to as chimerism. This is undesirable because as the plant grows, different tissues such as leaves and flowers will have different mutations, and interesting traits may not be inherited in the next generation. While this phenomenon has been known for many decades, precise studies at the genomic level are lacking. Understanding chimerism has practical applications because the tissue culture methods employed to remove chimerism are costly and time consuming. Further, any tissue culture beyond the point of chimera resolution is unnecessary work.

In 2012, the PBGL finalized research into the genetic effect of mutagenesis and chimerism in shoot apical meristems of banana. This work was published in the peer reviewed Plant Biotechnology Journal and was selected for the cover of the journal (Fig. 2). Careful study of the inheritance of induced mutations revealed that most measurable chimerism was removed within one month after treatment, and that mutations were fixed and heritable in all measured generations. This means that the time and effort of population development for mutation breeding of vegetatively propagated crops can be cut by half, making the process much more efficient. With these exciting findings, the PBGL is now testing this approach in other species, including vegetatively propagated cassava. To improve these studies, the PBGL plans to utilize its recently installed next generation sequencing facility. In 2012, the PBGL installed an Illumina MiSeq sequencer and associated support equipment that allows the sequencing of billions of DNA bases in a single machine run. The pilot experiment in cassava is being planned with just 8 mutant plants versus the approximate 800 banana plants that were used in the recently published work. This represents a large reduction in the time and labour needed for tissue culture.

In addition to genotyping activities aimed at making the process of mutation induction and population development more efficient, the PBGL continues its work in developing practical

and low-cost protocols that will work in laboratories in most developing countries. A key target is DNA extraction. DNA must be of high quality for use in downstream assays such as TILLING, molecular markers, genetic fingerprinting or sequencing. There are excellent kits on the market, but these kits are expensive and "home-made" methods tend to use toxic organic phase separation. This poses several problems, including the production of toxic waste that requires specialised disposal. Not all laboratories are properly equipped for the disposal of toxic organic compounds. In addition, improper phase separation leads to carry-over contaminants that inhibit downstream applications. To address these issues, the PBGL in 2012 developed a low-cost method for DNA extraction that does not use organic phase separation. In addition to reducing the toxicity while providing highly purified DNA suitable for a variety of assays (Fig. 3), the cost of this method per sample is a tenth of that of commercial kits. Validated in sorghum, barley and tomato, work is on-going to optimize the method for other crops. The finalized protocol is being distributed in 2013. The PBGL is also developing a DNA extraction positive control kit that can be shipped to Member States so that they can more easily adopt and adapt this methodology.

FIG. 3. A 2012 PBGL research fellow, Ms Mayada Beshir from Sudan, performing experiments to validate low cost and low-toxicity DNA extraction methods in different plant species.

Mutation detection – phenotyping

High-throughput phenotyping (often referred to as "Phenomics") has been identified as being a bottleneck in plant breeding and genetics. With the advancement of phenotyping technology the PBGL is aiming to acquire and establish enabling capacity in phenotyping to increase efficiency of mutant screening and accelerate delivery of mutant lines to Member States. The strategy is to develop and disseminate simple and user-friendly protocols for phenotyping that can easily be validated and adopted by Member States. Phenotyping varies depending on the target trait from a simple field or greenhouse trail to a more complex and expensive robotic imaging systems.

The PBGL has long worked on phenotyping for salt tolerance screening and expanded recently to other traits such as mutant seed characteristics. A priority for the PBGL is to develop and disseminate screening protocols to Member States for important biotic and abiotic stress-related traits such as pest and disease resistance and tolerance to drought, heat and salinity. In 2012, the PBGL completed a protocol for screening salt tolerance in rice. The salt tolerance protocol was further adapted to other cereal crops such as wheat and barley with the assistance of training fellows from Member States (Fig. 4a). Furthermore, the PBGL initiated work on developing a screen for drought tolerance in cereal mutants, in which training fellows from Member States participated (Fig. 4b).

FIG. 4a. Fellows (Mr Salimia and Mr Salama from the State of Palestine) learning the protocol for salt tolerance screening in wheat and barley. FIG. 4b. Fellows (Mr Hussaini from Afghanistan and Mr Ali from Bangladesh) taking observations from a drought-tolerance screen in barley.

Mutant line development – accelerated breeding

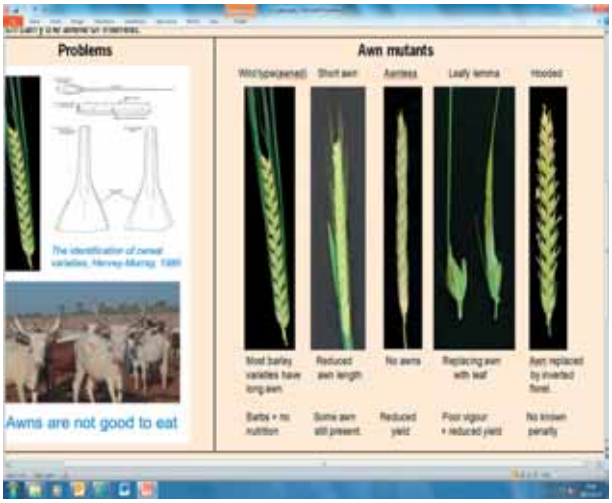

FIG 5. A normal barley spike (left) compared to potential awn mutants for greater fodder quality. The awn has no nutritious value and contains barbs that cause lacerations in the mouths of feeding animals; the removal of the awn is a major breeding objective. The most promising mutant is hooded as this is not associated with a yield penalty.

Prior to 2012, the PBGL focussed primarily on two major activities: 1) Mutation induction and 2) Mutation detection. Mutation induction takes a few seconds/ minutes and mutation detection takes a few months/years (though this is getting faster with high-throughput methods). The biggest constraint in plant mutation breeding is the time taken to develop selected mutants into crop varieties; this can take 10 years for an annual crop. There is an increasing urgency and demand by Member States to produce and market improved mutant varieties as soon as possible. In order to address this need the PBGL has initiated a new (third) activity area, accelerated breeding for mutant traits. The aim is to reduce the time to release mutant crop varieties. Accelerated breeding is achieved through the combined technologies of: 1) trait selection (desired mutants); 2) development of diagnostic markers for the mutant trait; 3) genetic fingerprinting to select for the elite genetic backgrounds; 4) rapid generation cycling to induce early flowering; and 5) tissue culture methods such that shortcut the selection of desired lines. These methods can also be used to transfer a desired mutant trait from one line into another, thus speeding up the dissemination of useful mutant traits. In 2012 the PBGL evaluated a series on barley mutants of value in fodder quality and initiated work on the rapid introduction of the hooded and low lignin mutants to improve palatability and digestibility, respectively (Fig. 5). The first crosses of the mutant stocks were made onto the recipient varieties and the first backcrosses were made. The time between generation times was more than halved (from 85 to 35 days) using small pots, continuous lighting and the culture of three week old embryos.. In addition genetic markers were designed for both mutant genes, which can be used to screen progeny that possess the desired mutant traits.

CAPACITY BUILDING AND SERVICES

The PBGL has a dynamic training programme. We receive individual fellows and groups for training. The details of training activities carried out in 2012 are given below.

Individual fellowship training

Name	Country	Duration	Training topics
Ali, Mohammad	Bangladesh	3 months	Mutation induction and detection, with special reference to abiotic stress tolerance. Training done in collaboration with Soil and Water Management and Crop Nutrition Laboratory
Beshir, Mayada	Sudan	4 months	Mutation induction and detection, with a special reference to development of low-cost assays for molecular characterization of mutant sorghum.
Hussaini, Sekander	Afghanistan	4 months	Enhancing crop productivity through mutation breeding for biotic and abiotic stresses, with special reference to wheat
Mandakombo, Noeall Benedicte	Central African Republic	1 month	Methods in induced mutation of crop plants

Group training

One-week group training was conducted on *Developing Germplasm through TILLING in Crop Plants using Mutation and Genomic Approaches*, 25-29 June, 2012 (TC project PAK5047)

Name	Country
Abro, Saifullah	Pakistan
Ali, Akhtar	Pakistan
Shokat, Sajid	Pakistan
Ur Rahman, Mehboob	Pakistan

Group fellowship training

A regional training course was conducted on *Methodologies and mechanisms for screening against stress*, 4-8th June 2012.

Name	Country
Abod, Majeid	Syrian Arab Republic
Al-Doori, Waleed	Iraq
Al-Gayyar, Adel	Iraq
Al-Mahdawi, Hussein Mohammad	Iraq

Name	Country
Almuwalld, Mohammed	Saudi Arabia
Al-Raisi, Ibtihal	The Sultanate of Oman
Haddadin, Maisa'a	Jordan
Hussaini, Sekander	Afghanistan
Khashoggi, Abdulmajeed	Saudi Arabia
Madadha, Afaf	Jordan
Raad, Faten	Lebanon
Tahier, Nawrez	Syrian Arab Republic
Thabet, Mansour	Yemen
Zaid, Nagi	Yemen

Scientific visitors

Name	Country	Areas of training	Period
Olu-Wakemi, Catherine Esoula	Nigeria	Induced mutation, molecular markers and TILLING in vegetatively propagated banana and fluted pumpkin	7-8 May 2012
Yadamsuren, Myagmarsuren	Mongolia	Mutation breeding in wheat, drought tolerance	16-17 August 2012
Salimia, Rezq	State of Palestine	Initiation of mutation breeding and screening for stress tolerance	1-15 October
Salimia, Ayed	State of Palestine	Initiation of mutation breeding and screening for stress tolerance	1-15 October
Omosun, Garuba	Nigeria	Mutation induction in vegetable crops	1-30 October 2012
Luyindula, Ndiku Sebastien	Democratic Republic of Congo	Mutation breeding technologies	5-14 November 2012

Consultants

Name	Country	Areas of expertise	Period
Rusfiandi, Heru	Indonesia	Pollen irradiation	15 May – 16 November 2012
Huang, Biguang	China	Mutation breeding	1 July – 31 December 2012

Radiation services

FIG. 6. Group fellowship training in plant mutation breeding.

The Seibersdorf Laboratories has a newly refurbished gamma-cell and an X-ray irradiator, which are available to Member States requiring plant mutagenesis. Requests for radiation services increased in 2012; 37 requests were received from 21 Member States for 23 plant species (details are given below). Most requests were for gamma irradiation, but there were also requests specifically for X-ray irradiation. In many cases we perform a radio-sensitivity test to determine the optimal irradiation dose for mutation induction; in other cases the requestor has specific dose requirements.

The following Irradiation services were provided by PBGL during 2012:

Member State	Crop species
Afghanistan	Wheat
Bangladesh	Jute
Botswana	Cowpea and maize
Burkina Faso	Sorghum
Central African Republic	Cassava
Eritrea	Barley
India	*Withania somnifera*
Indonesia	Rice
Italy	Strawberry
Kenya	Cowpea, sorghum and wheat
Kuwait	Barley
Mongolia	Barley and wheat
Netherlands	Ornamentals (*Argyranthemum, Geranium, Persicaria* and *Helenium)*
Nigeria	Okra
Poland	Lupin
Spain	Arabidopsis
Sudan	Sorghum and wheat
United Kingdom	Barley, wheat and ornamentals (*Alcea rosea, Ipomea purpurea* and *Petunia multiflora*)

Member State	Crop species
USA	*Acer truncatium*
Yemen	Garlic
Zimbabwe	Cowpea (*Vigna ubuiculata* and *V. subterranea*) and groundnut

Member State information sheets

The Fellows who come for training at the PBGL come to learn techniques in plant mutation breeding: mutation induction, mutation detection and mutant line development. They come in order to apply these techniques to problems limiting yield in their home countries. However, they also come with success stories. We ask our Fellows to produce an "Information Sheet for Visitors" that describes problems in agricultural production for their country, the traits targeted for crop improvement, success in producing mutant varieties, training received at the PBGL and collaborations with the Joint FAO/IAEA Division. In 2012 ten "Information Sheets for Visitors" were produced: for Afghanistan, Bangladesh, China, Indonesia, Mongolia, Nigeria, Peru, Thailand, Turkey and USA.

FIG. 7. Information Sheets for Visitors: the 2012 collection.

PUBLICATIONS

Books

Plant Mutation Breeding and Biotechnology (2012). Edited by Q.Y. Shu, B.P. Forster and H. Nakagawa. CABI, pp. 608.

This book covers the underlying scientific principles, state-of-the-art technologies and methodologies of plant mutagenesis. It describes historical developments and commonly used methods and terms in mutation induction, mutation detection and mutation breeding. It is a reference book suitable for students and professionals in practical plant breeding as well as in plant breeding research and development. The book includes case studies and examples and an appendix of recommended irradiation treatments for nearly 200 plant species.

Peer-reviewed publications

Jankowicz-Cieslak, J., Huynh O.A., Brozynska, M., Nakitandwe, J., Till, B.J. (2012). Induction, rapid fixation and retention of mutations in vegetatively propagated banana. Plant Biotechnology Journal 10: 111.

Kozak, K., Jankowicz-Cieslak, J., Bado, S., Till, B.J., Galek, R., Sawicka-Sienkiewicz, E. (2012). Inter-varietal differences of *Lupinus angustifolius* in response to chemical and physical mutagens. In: Naganpwska, B., Kachlicki, P., Wolko, B. (Eds.). Lupin crops – an opportunity for today, a promise for the future. Proceedings of the 13th International Lupin Conference, 6-10 June 2011, Poznań, Poland. International Lupin Association, Canterbury, New Zealand. ISBN 978-83-61607-73-1: 112-117.

Maghuly, F., Jankowicz-Cieslak, J., Till, B., Laimer, M. (2012). The use of ECO-TILLING for the genetic improvement of *Jatropha curcas*. In: Sujatha, M., Bahadur, B., Carels N., Compendium of Bioenergy Crops: *Jatropha curcas*. Science Publishers, USA.

Wening, S., Croxford, A.E., Ford, C.S., Thomas, W.T.B., Forster, B.P., Okyere-Boteng, G., Nelson, S.P.C., Caligari, P.D.S., Wilkinson, M.J. (2012). Ranking the value of germplasm: new oil palm (*Elaeis guineensis*) breeding stocks as a case study. Annals of Applied Biology 160: 145-156.

Wang, T., Uauy, C., Robson, F., and Till, B. (2012). Tilling *in extremis*. Plant Biotechnology Journal 10(7):761-72.

Till, B.J., Zerr, T., Comai, L., Heinkoff, S. (2012). A protocol for TILLING and eco-TILLING . In: Shu, Q.Y., Forster, B.P., Nakagawa, H. (Eds.). Plant Mutation Breeding and Biotechnology, chapter 22, pp. 269-286.

Cooper, J.; Henikoff, S.; Comai, L., Till, B.J. (2012). TILLING and Ecotilling for Rice. In: Methods in Molecular Biology: Rice Protocols. Springer, pp. 39-56.

Jankowicz-Cieslak, J.; Huynh, O.A.; Dussoruth, B.; Saraye, B., Till, B.J. (2012). Low cost mutation discovery methods suitable for developing countries. ScienceMED, vol.3/3: 245-249.

Conference abstracts

Kyaw, M.T., Bado, S., Matijevic, M., Huynh, O.A., Myint, T.T. (2012). Phylogenetic analysis of Myanmar rice and its mutant varieties. Myanmar Health Research Congress 2011, 9-13 January, 2012.

Jankowicz-Cieslak, J., Dussoruth, B., Forster, B.P., Till, B.J. (2012). Characterization of *Musa* Germplasm using Low-cost SNP and Indel Discovery. International Conference on Molecular Mapping & Marker Assisted Selection, Vienna, Austria, 8-11 February 2012.

Maghuly, F., Ramkat, R., Taassob-Shirazi, F., Jankowicz, J., Laimer, M. (2012). Analysis of genetic variation among and within *Jatropha* species using dominant markers. In: Molecular Mapping & Marker Assisted Selection, 8-11 February 2012, Vienna, Austria

Huynh, O.A., Jankowicz-Cieslak, J., Kozak-Stankiewicz, K., Dussoruth, B., Cui, H., Forster, B.P., Till, B.J. (2012). Developing low cost mutation discovery methods suitable for developing countries. International Conference on Molecular Mapping & Marker Assisted Selection, Vienna, Austria 8-11 February 2012, pp. 27.

Till, B.J., Jankowicz-Cieslak, J., Huynh, O.A., Bado, S., Forster, B.P. (2012). Mutation based approaches for functional genomics in vegetatively propagated plants. International Conference on Molecular Mapping & Marker Assisted Selection, Vienna, Austria, 8-11 February 2012, pp. 28.

Matijevic, M., Lagoda, P.J.L., Franckowiak, J.D., Forster, B.P. (2012). Evaluation of barley mutants for improved fodder production. International Conference on Plant Growth, Nutrition & Environmental Interactions, Vienna, Austria, 18-21 February 2012, pp. 80.

Jankowicz-Cieslak, J., Scharl, T., Brozynska, M., Adu-Gyamfi, J., Forster, B.P., Rapa, M. (2012). Diversity in Physiological Responses to Drought by *Musa* Genotypes. International Conference on Plant Abiotic Stress Tolerance II, Vienna, Austria, 22-25 February 2012, no. 167.

Bado, S., Adu-Gyamfi, J., Forster, B.P., Laimer, M. (2012). Ion Accumulation in Rice Genotypes varying in Salt Tolerance. International Conference on Plant Abiotic Stress Tolerance II, Vienna, Austria, 22-25 February 2012, no. 168.

Maghuly, F., Jankowicz-Cieslak, J., Ramkat, R.C., Till, B.J., Laimer, M. (2012) Reverse genetics and dominant markers to determine the genetic variation in *Jatropha curcas*. In: Plant and Animal Genomes XX Conference, San Diego, USA, 14-18 January 2012, San Diego, USA, abstract P0731.

Till, B.J., Jankowicz-Cieslak, J., Huynh, O.A., Bado, S., Henry, I., Comai, L., Forster, B.P. (2012). Mutation induction and reverse-genetics for vegetatively propagated plants. In: Plant and Animal Genomes XX Conference, San Diego, USA, 14-18 January 2012, San Diego, USA, abstract W450. Till, B.J., Jankowicz-Cieslak, J., Maghuly, F., Huynh, O.A., Bado, S., Forster, B.P., Laimer, M. (2012). Mutation based approaches for functional genomics and trait development in vegetatively propagated plants. In: Plant and Animal Genomes XX Conference, San Diego, USA, 14-18 January 2012, San Diego, USA, abstract W547.

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); seed stocks.
International Network for the Improvement of Banana and Plantains (INIBAP), Biodiversity International, Montpellier, France.	Induced mutations in *Musa* for tolerance to biotic stresses and development and deployment of genomics tools for the crop.
Austrian Institute of Technology, Health & Environment Department (Dr Silvia Fluch, Dr Kornel Burg), Tulln, Austria.	Gene expression profiling in drought stages.
Institute for Biotechnology in Plant Production, University of Natural Resources and Life Sciences, (BOKU; Prof. Herman Buerstmayer, Dr Hans Vollmann, Dr Heinrich Grausgruber), Tulln, Austria.	Fellowship training. Near-infrared spectrometry analysis in characterising mutant seed phenotypes. Accelerating plant breeding.
University of Agriculture, Department of Plant Physiology (Dr Marcin Rapacz), Krakow, Poland.	Banana phenotyping for drought tolerance.
University of Natural Resources and Life Sciences, Department of Biotechnology (Prof. Margit Laimer, Dr Fatemeh Maghuly), Vienna, Austria.	Induced and natural mutations detection in understudied crops.
University of Natural Resources and Life Sciences, Department of Biotechnology (Dr Theresa Scharl), Vienna, Austria.	Statistical data evaluation and treatment.
Agri-Science Queensland, Hermitage Research Facility (Prof. Jerome Franckowiak), Warwick, Australia.	Barley crossing protocol; barley mutants and mutation breeding.
The James Hutton Institute (Dr William Thomas), Invergowrie, Scotland, UK.	Barley crossing protocol, field experimentation, barley genetic stocks, plant breeding.
Nordic Genetic Resource Center (Dr Udda Lundqvist), Alnarp, Sweden.	Classic barley mutants, mutant gene descriptions and mutant nomenclature. Seed stocks.

Institution	Topic
University of California Davis Genome Center, (Prof. Luca Comai, Dr Isabelle Henry), Davis, California, USA	Developing next generation sequencing strategies for discovery of induced mutation events in genomes of vegetatively propagated crops.

THE SOIL AND WATER MANAGEMENT AND CROP NUTRITION LABORATORY

EXECUTIVE SUMMARY

The aim of the Soil and Water Management and Crop Nutrition Laboratory (SWMCNL), as an integral part of the SWMCN Subprogramme, is to develop, adapt and deliver a range of soil, water and crop management technology packages and practices to Member States using isotope and nuclear techniques.

In 2012, the SWMCN Laboratory provided a broad range of services related to: (i) the development and validation of robust and affordable isotope and nuclear techniques for climate-smart agriculture; (ii) the training of technical staff and scientists from Member States in the analyses of isotopes and the use of nuclear and related techniques to develop improved and integrated soil-nutrient-water-plant management practices; (iii) the provision of isotope analyses to Member State institutions where analytical facilities are not available; and (iv) the provision of quality assurance services to Member States.

Research and development activities at the SWMCNL focused on the design of multiple robust and affordable isotope and nuclear techniques to improve soil and water management in climate-smart agriculture. This included the validation of oxygen-18 isotope techniques for quantifying soil evaporation and plant transpiration to improve water use efficiency in rainfed and irrigated agriculture. In addition, further improvements were made in the use of carbon-13 and nitrogen-15 isotopes for assessing soil organic carbon sequestration under on-farm conditions, and beryllium-7 as a tracer for short-term erosion events. Methods were also adapted to investigate the use of oxygen-18 in phosphate to trace phosphorus sources and cycling in soils and ultimately to provide a better understanding of soil phosphorus dynamics in agro-ecosystems. Collaboration with the IAEA's Nuclear Spectrometry and Applications Laboratory has been initiated to compare the precision of in-situ fallout radionuclide measurements in the field with those of conventional laboratory based gamma-ray spectrometry to assess soil erosion. All these activities have made important progress and are essential in the implementation of several Coordinated Research Projects of the SWMCN Subprogramme.

A second major activity of the SWMCNL is its contribution to capacity building in Member States. The laboratory hosted 16 fellows and one intern, each receiving about three months' intensive training in the application of isotopic and nuclear techniques to improve soil and water management and crop nutrition practices. The SWMCNL also conducted a one-month training course entitled "Soil and Water Management in Agriculture to Support Crop Production in Asia and the Pacific" for 20 scientists and technicians from 16 countries across the Asia and Pacific region. This training course was funded by the Government of Japan under IAEA's Peaceful Uses Initiative (PUI). The Laboratory also organized an internal training course funded by the Joint FAO/IAEA Division on the use of internet-based geospatial information visualization tools to disseminate and promote the work carried out by the Joint FAO/IAEA Division.

Two detailed protocols on the use of isotope techniques for improving soil conservation strategies were prepared and are expected to be published in 2013. The SWMCNL published 31 articles, of which 12 were in international peer-reviewed journals and 15 presented during the FAO/IAEA International Symposium on "Managing Soils for Food Security and Climate Change Adaptation and Mitigation", 23-27 July 2012, Vienna, Austria.

A total of 4757 and 145 samples were analysed at the SWMCNL for stable isotopes and fallout radionuclides, respectively. Most analyses were carried out to support research and development activities at the SWMCNL, focusing on the design of multiple robust and affordable isotope and nuclear techniques to improve soil and water management in climate-smart agriculture.

STAFF

Name	Title
Dercon, Gerd	Laboratory Head
Adu-Gyamfi, Joseph	Soil Scientist/Plant Nutritionist
Mabit, Lionel*	Soil Scientist
Mayr, Leo	Senior Laboratory Technician
Arrillaga, José Luis	Senior Laboratory Technician
Aigner, Martina	Senior Laboratory Technician (50%)
Heiling, Maria	Senior Laboratory Technician (50%)
Toloza, Arsenio	Laboratory Technician
Resch, Christian	Laboratory Technician
Jagoditsch, Norbert	Technical Attendant
Augustin, Franz	Technical Attendant
Mletzko, Joanna Malgorzata	Team Assistant

* *Left the SWMCNL in February 2012*

MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The Soil and Water Management and Crop Nutrition Laboratory (SWMCNL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture plays a key role in the implementation of the Soil and Water Management & Crop Nutrition (SWMCN) Subprogramme. The SWMCNL assists in the development and transfer of isotope and nuclear

techniques to Member States to optimize soil, water and nutrient management practices and strategies for sustainable agriculture with high resilience to climate change, and contributes to improved crop water use efficiency, soil organic carbon sequestration and reduced greenhouse gas emissions from farmlands.

With an increasing focus in Member States to enhance the efficient use of soil and water resources and improve the ecosystem services of these, there are significant shifts in the use of isotope and nuclear techniques from a field plot approach to an area-wide scale. The development and adaptation of new, more robust, cheaper and cost-effective isotope and nuclear techniques make it possible to effectively measure changes in soil and water quantity and quality at a range of scales under real-time and on-farm conditions (Fig. 1). The use and analysis of isotopes is therefore no longer constrained to laboratory operating conditions. This shift makes isotope and nuclear techniques more accessible to Member States, including those with limited technical and analytical capacity.

The SWMCN Laboratory provides a broad range of services driven by Member States' demands. These include:

FIG. 1. Robust and real-time isotope and nuclear techniques allow scientists and technicians to effectively measure changes in water use efficiency at a range of scales under real-time and on-farm conditions.

- Development and validation of isotope and nuclear techniques for use in Coordinated Research Projects (CRPs) and Technical Cooperation Projects (TCPs). About ten isotopic and nuclear techniques have been developed or adapted in the SWMCNL over the last 50 years, which are now well established across the world. Currently, about five techniques are under development;
- Training of scientists and technicians from Member States in the analyses of isotopes and the use of nuclear and related techniques to develop improved and integrated soil-nutrient-water-plant management practices (through individual fellowships, group trainings and training courses);
- Provision of isotope analyses to projects counterparts where analytical facilities are not available;
- Provision of quality assurance services to Member States.

The SWMCNL supports the following activities:

- Identify farm management factors affecting fertilizer and water use efficiency in both rainfed and irrigated agriculture;
- Quantify contribution of nitrogen from organic sources to crop nutrition and biological nitrogen fixation;

- Assess the effectiveness of soil and water conservation strategies in controlling soil erosion and improving water quantity and quality.

Vacuum extraction of soil and plant water for stable isotope analyses

The need for a rapid and inexpensive technique for routine oxygen-18/oxygen-16 ($^{18}O/^{16}O$) extraction of water from plant and soil samples is increasing due to the greater demand for isotopic data in agro-ecological, soil water and crop water use studies, essential for improving water use efficiency in rainfed and irrigated agriculture. The commonly used water extraction techniques are often laborious, time consuming and involve complicated setup with custom-made glass apparatus. In addition, liquid nitrogen or dry ice is needed to freeze and trap water vapour that evaporates during extraction. However, both of these cooling agents can be difficult to acquire in many developing countries. With water isotope analyses becoming cheaper, easier and faster (through the development of the modern laser isotope analyser such as Cavity Ring Down Spectroscopy (CRDS)), the bottleneck in sample throughput is often the water extraction time and not the isotopic analysis of water.

Here we describe results obtained using a simple, fast and accurate vacuum distillation method using a commercial immersion cooler and a vacuum container filled with 2-propanol at -50°C instead of the liquid nitrogen or dry-ice for freezing water vapour. The method can be easily adopted at a relatively low cost and allows large numbers of samples to be extracted quickly for isotopic analyses.

The methodology was successfully tested with two soil types (a sandy loam and a silty clay loam), over two water levels (near field capacity and near wilting point) and over a range of extraction times (15, 30, 60, 120 and 180 minutes). The results showed that the water recovery (extracting more than 98% of the total water) for sandy soil under wet conditions is as low as 15 minutes versus 30 minutes in dry soil; in clay soil it was 30 versus 120 minutes under wet and dry conditions, respectively. The complete configuration (see Fig. 2) provides a rapid, low-cost and reliable method that meets the high-throughput requirements for leaf and soil water isotopic analyses.

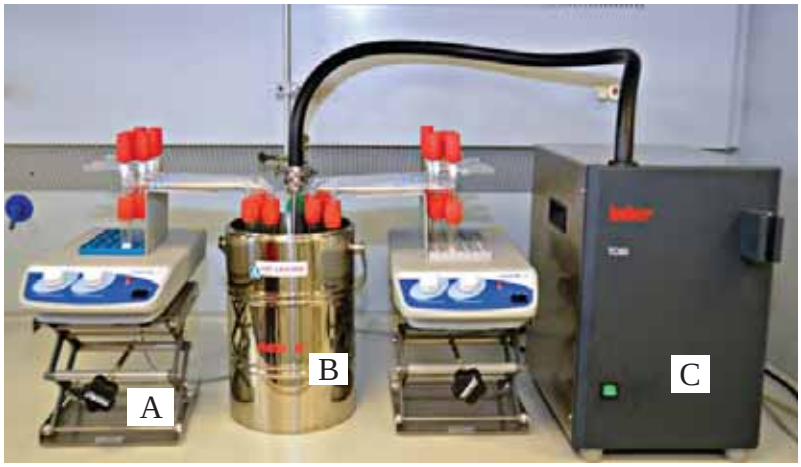

FIG. 2. An improved vacuum distillation method for extracting water from soil and plant for oxygen-18 analyses, with heating blocks (A), vacuum container filled with 2-propanol (B) and immersion cooler (C).

Validating the components of evapotranspiration from soil evaporation and plant transpiration, with oxygen-18 isotopes and micrometeorology

Agriculture is the single largest consumer of freshwater, acounting for 70% of water use in most parts of the world. However, global water use efficiency is less than 40%. There is therefore an urgent need to improve agricultural water use efficiency through an increase in crop water productivity, i.e. the productivity of crop per unit of total water consumption. However, information on crop water productivity and transpiration efficiency, i.e. the crop biomass per unit of transpired water under different irrigation technologies, and the extent and proportion of evapotranspiration (ET) as soil evaporation (E) and crop transpiration (T) under different agro-climatic and soil-plant management conditions are often unavailable.

Loss of water from the soil surface through evaporation is often a major component in the soil water balance of agricultural systems in semi-arid regions. Studies show that estimates of soil evaporation can range between 30% to more than 60% of the seasonal rainfall. The contribution of evaporation to total water loss needs to be quantified so that management practices can be devised to reduce this loss and improve water use efficiency.

Not many methods are available to quantify this evaporation component. Simple stable isotopic tracer methods have been used to separate evaporation and transpiration components. The distinctions in isotopic composition between soil evaporation and plant transpiration and that of background water vapour around the vegetation allow the contributing fractions of evaporation and transpiration to total water loss through evapotranspiration to be estimated by generating the isotopic turbulent mixing relationships.

A study was carried out on a wheat crop over a three-day period in April at the experimental field of the University of Natural Resources and Life Sciences, Vienna, to provide an independent check of the relative proportions of soil evaporation and plant transpiration estimated by the isotopic method and by the canopy turbulence model[3] of water vapour transport in plant canopies using the conventional eddy covariance method. The results showed that the transpiration rates, estimated by the $\delta^{18}O$ isotopic technique, were similar to those derived from canopy turbulence analyses (Fig. 3), indicating that the two methods gave essentially the same partitioning of evapotranspiration into evaporation and transpiration. This shows that isotope based techniques can complement or validate information obtained by conventional techniques. This information is needed for many purposes including predicting evapotranspiration, modelling soil nutrient transformation processes such as nitrification and denitrification, and examining the response of plants to water stress.

[3] The canopy turbulence model, which assists with inverse Lagrangian dispersion analysis, identifies sources and sinks of water vapour and CO_2 and other gases in forests and crops.

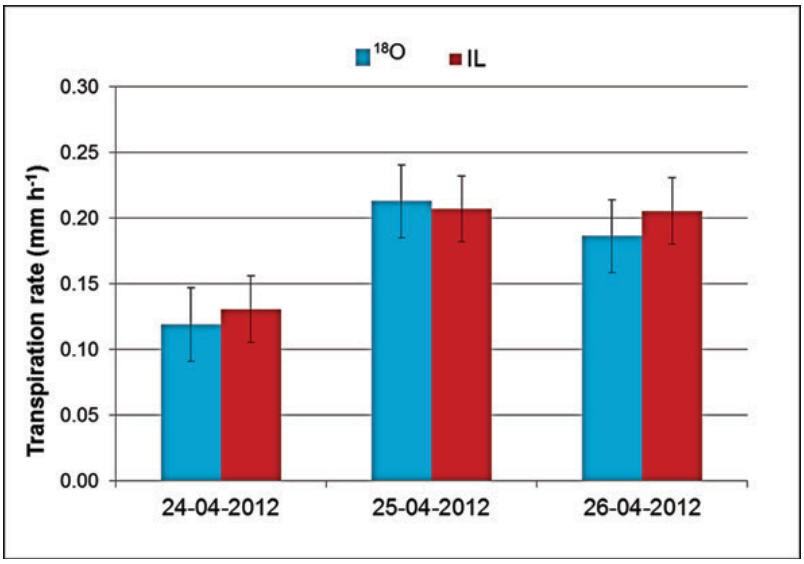

FIG. 3. Average daytime transpiration rates determined by $\delta^{18}O$ and inverse Langrangian (IL) canopy turbulent analyses.

Assessment of carbon distribution and soil organic carbon storage in mulch-based cropping systems by using isotopic techniques

The quest for rapid and cost-effective techniques to assess carbon distribution and soil organic carbon storage led to the development of isotopic techniques based on the use of the nitrogen-15 and carbon-13 natural abundance signal of bulk soil and soil organic matter pools.

In 2012, the SWMCNL made further improvements in the use of nitrogen-15 isotopes (natural abundance) for assessing soil organic carbon stability in farmland. In long-term field experiments, established on soils poor and rich in soil organic matter and in close collaboration with the Austrian Agency for Health and Food Safety and the University of Natural Resources and Life Sciences, Vienna, the nitrogen-15 signature of different soil organic matter fractions was used in combination with their carbon:nitrogen ratio to derive a proxy for soil organic carbon stability. The concept is based on the increase in $\delta^{15}N$ and the decrease in carbon:nitrogen ratio with increasing carbon stability, which has been developed for grasslands at different elevations in the Swiss Alps by Conen *et al.* (2008)[4].

Fig. 4 shows the obtained results for Chernozem soils, rich in soil organic carbon, in Groß-Enzersdorf. Very similar values and trends were found for the very different agro-ecosystem of Grabenegg, with poorer and wetter Cambisol soils in the transition zone towards the Austrian Alps. Based on these preliminary analytical results of Grabenegg and Groß-Enzersdorf, we expect that the Conen approach may be promising not just for grasslands, but also for intensively used farmland. Depending on tillage and cropping system and on soil depth, it predicted mineral-associated organic carbon to be 53 to 930 times more stable than particulate organic carbon, with increasing stability in the deeper subsoil. These results are now being

[4] Conen, F., Zimmermann, M., Leifeld, J., Seth, B., Alewell, C. (2008). Relative stability of soil carbon revealed by shifts in delta N-15 and C:N ratio. Biogeosciences, 5:123-128.

validated by carbon-14 dating in collaboration with the University of Vienna and the ETH-Zürich (Eidgenössische Technische Hochschule) in Switzerland. Further sites in Western Europe and Sub-Saharan Africa will be tested in 2013 and 2014.

More information on the context of this research can be found in the video on *More Food With Better Soil – Using Isotope Techniques To Improve Soil Quality* (*http://www-naweb.iaea.org/nafa/swmn/index.html#*), prepared in the context of the IAEA Scientific Forum on "*Food for the Future: Meeting the challenges with Nuclear Applications*", held on 18 September during the 2012 IAEA General Conference.

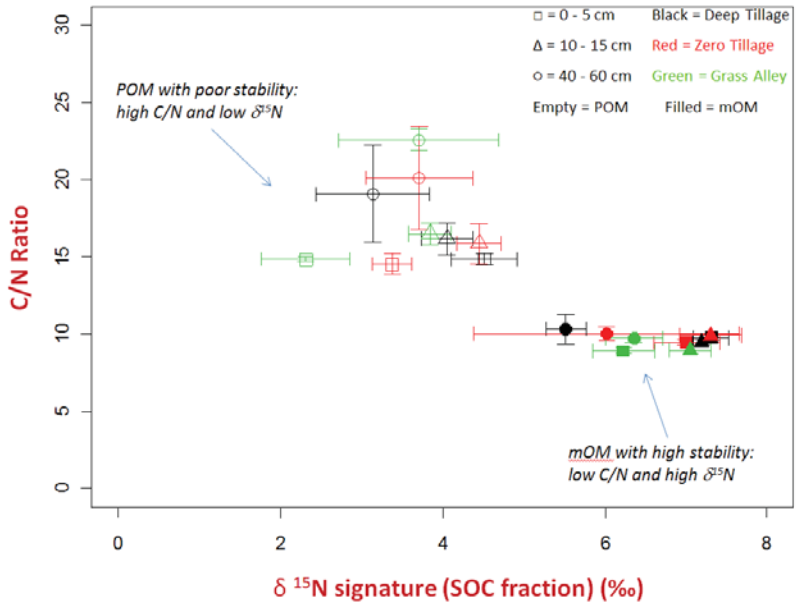

FIG. 4. Carbon:nitrogen ratio versus δ ^{15}N signature of different soil organic carbon fractions under different tillage and cropping systems at different depths (Chernozem Soil at Groß-Enzersdorf, Austria).

Use of oxygen-18 isotopes in phosphate to trace phosphorus sources and cycling in soils

Phosphorus is a key nutrient essential for all livings organisms. Soils with low phosphorus status are widespread in many regions of the world where phosphorus deficiency limits plant growth and reduces crop production and food quality. Phosphorus has one stable isotope (phosphorus-31) and several radioisotopes (from phosphorus-26 to phosphorus-30 and from phosphorus-32 to phosphorus-38), but the only two isotopes (phosphorus-32 and phosphorus-33) that are suitable for agronomic studies have a very short half-life, making them unsuitable for long term studies. Because phosphorus has only one stable isotope, researchers have started to explore the potential of the oxygen-18 isotope in inorganic phosphorus compounds to study and understand phosphorus dynamics in both cropping and livestock production systems for improving soil phosphorus fertility and food productivity.

In order to analyse $\delta^{18}O\text{-}PO_4$, phosphate in soils from different soil phosphorus fractions must be extracted from the soil, purified and converted to Ag_3PO_4. Tamburini *et al.* (2010)[5] have developed protocols for estimating $\delta^{18}O\text{-}PO_4$ in soils with different phosphorus status and plant availability in different countries. Soils subjected to different farm management practices (e.g. fertiliser or manure applications) show different $\delta^{18}O\text{-}PO_4$ signatures, indicating the potential as isotopic tracer for studying phosphorus cycling, tracing phosphorus sources and ultimately providing a better understanding of soil phosphorus dynamics in agro-ecosystems.

Recognizing the importance of increasing phosphorus use efficiency to improve land productivity in tropical and subtropical regions, particularly with rapid reduction of global phosphorus reserves, the SWMCNL initiated in 2012 a collaboration with scientists from China and Switzerland to test this new technique based on the use of the oxygen isotope composition of phosphate, $\delta^{18}O\text{-}PO_4$ (Fig. 5), to trace phosphorus in soils and plants under on-farm conditions. The new analytical technique is now available in the SWMCNL and will be further tested in 2013 on rock phosphate materials from across the world.

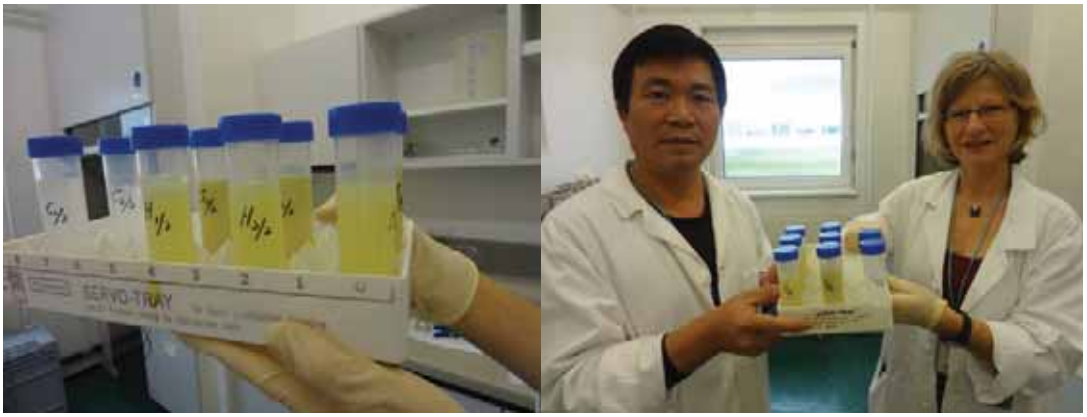

FIG. 5. Testing a new stable isotope-based technique to trace phosphorus in soils and plants

Beryllium-7 as a short-term radiotracer of soil movement: potential and limitations

These experiments, carried out in Seibersdorf, Austria, aimed to assess the potential and limitations of beryllium-7 as a short-term radiotracer of soil movement.

Assessing the influence of heavy rains on Beryllium-7 distribution in soils under field conditions

The beryllium-7 technique used in soil erosion research is based on the assumption that the pre-existing beryllium-7 and inputs associated with heavy rains are uniformly spatially distributed in the study field and reference sites. In the current study, soil cores were collected to a depth of 4 cm from a previously cultivated level site after the occurrence of heavy rains (>20 mm d^{-1}) in order to assess the beryllium-7 spatial distribution. A fine soil increment

[5] Tamburini, F., Bemansconi, S.M., Angert, A., Weiner, T., Frossard, E. (2010). A method for the analysis of the δ18O of inorganic phosphate extracted from soils with HCl. European Journal of Soil Science, 61:1025–1032.

collector was used for the soil sampling, a portable device specially developed to collect very fine soil layers. The beryllium-7 depth profile in Seibersdorf soil showed an exponential decrease (see Fig. 6), indicating that the field had not been disturbed since the last tillage in autumn 2011. Beryllium-7 could be found only in the upper 25 mm of soil, with an average value of 312 Bq m^{-2} and a coefficient of variation (CV) of 19%. This CV of 19% is acceptable for a level field with no evidence of soil movement after the occurrence of heavy rainfalls and indicated that the heavy rains did not affect the uniformity of the spatial distribution of beryllium-7 at the experimental site.

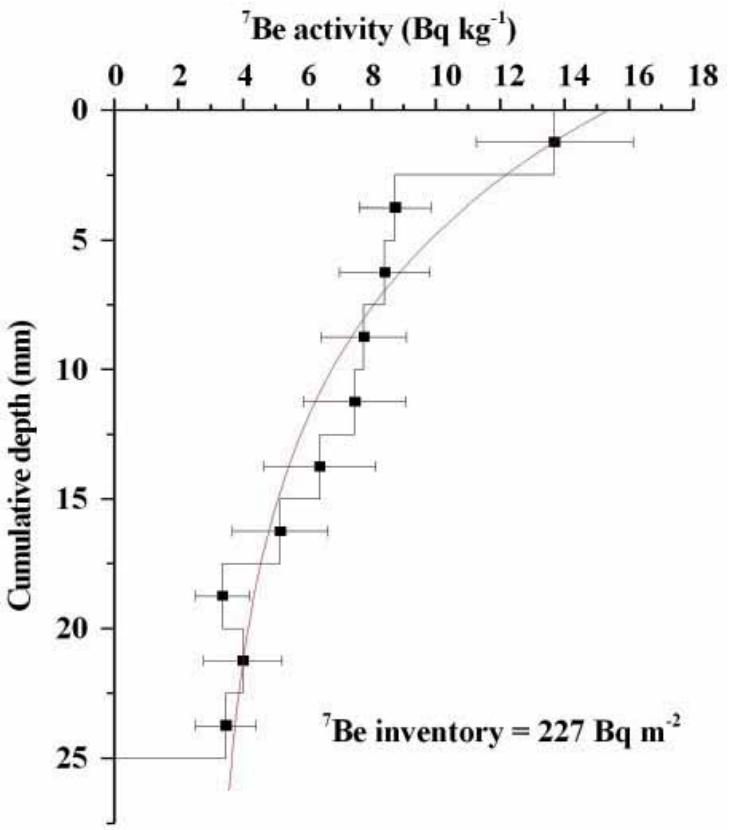

FIG. 6. The vertical distribution of beryllium-7 in soil, showing error bars at the 95% confidence level.

Using beryllium-7 in soil erosion assessments in partially covered soils

One of the main limitations when using the beryllium-7 approach for soil erosion studies is the interception of beryllium-7 by the plant canopy in agro-ecosystems. Thus, the application of this radiotracer for estimating soil erosion rates has been limited to bare soils without any vegetation. The current research was focused on the assessment of the influence of above-ground biomass on the use of beryllium-7 for short-term soil erosion studies in agricultural land. The study was performed in collaboration with the Terrestrial Environmental Laboratory in Seibersdorf. The objectives of the study were: (i) to determine the beryllium-7 interception fraction (i.e. the proportion of ^{7}Be in rain inputs intercepted) and accumulation of bean leaves in the early growing stage (15 days after sowing) and (ii) to assess the beryllium-7 root-to-foliar transfer. As the beryllium-7 fallout is mainly deposited through precipitation, greenhouse plots with the young bean plants were put outside to allow beryllium-7 deposition during

rainfall (Fig. 7). Beryllium-7 concentrations were determined in bean leaves, roots, soil, rainwater and air in order to obtain a complete inventory of beryllium-7 from all sources. Preliminary results showed that bean canopies with high soil coverage have high capacity to intercept and accumulate beryllium-7, but that the interception fractions decreased with increasing amount of rain.

FIG. 7. Sampling common bean leaves in the greenhouse for ^{7}Be gamma measurements.

The characteristics of the rainfall, the elemental properties of the radionuclide and the bean coverage factor were primarily responsible for the retention of beryllium-7 by the plants. The mass interception factor, which describes the retention of beryllium-7 by the plant caused by wet deposition, was calculated to be about 3.8 m^2 kg^{-1} (for a 0.4 mm rain 15 days after sowing). The beryllium-7 concentration in air decreased by 77%, from an initial value of 10.12 mBq m^{-3} to 2.33 mBq m^{-3} after two consecutive rainfalls of different intensities and quantities (0.4 mm and 7.2 mm). No beryllium-7 could be found in the bean roots ten days after rainfalls had occurred. It was therefore concluded that no root-to-foliar transfer of beryllium-7 had taken place due to the shortage period of time and to the limited availability of beryllium-7 in the soil. More experiments are needed for different types of crops in order to validate these findings.

Assessing soil erosion at landscape level by means of the fallout radionuclides method via in-situ gamma spectrometry

FIG. 8. Imagery data (Google Earth Pro; dated 03/14/2002) showing aerial (top) and eye level (bottom) views of the experimental field site. Red markers indicate GPS coordinates of measurement and reference sites.

Research and development activities were started in 2012 to assess soil erosion using *in-situ* gamma spectrometry to measure fallout radionuclides. *In-situ* gamma spectrometry can give near instantaneous results, allowing prompt decisions to be made and identification of critical spots of soil erosion whilst the equipment is still in the field. This offers significant advantages over laboratory based gamma spectrometry, which, although it is more sensitive, has a lengthy measuring time (approx. 1 day).

Our objective was to establish the correlation between the *in-situ* field and the laboratory based measurements, and to develop new strategies for landscape based soil erosion

assessment. This work was carried out in collaboration with the Nuclear Spectrometry and Applications Laboratory in Seibersdorf. Laboratory measurements were made by a p-type coaxial HPGe semiconductor detector, whereas the *in-situ* measurements were made by a lanthanum bromide ($LaBr_3$ (Ce)) scintillation detector.

The study was carried out in Grabenegg, at the experimental research station of the Austrian Agency for Health and Food Safety. Grabenegg is west of Vienna, between the towns of Melk and Wieselburg. It is at an altitude of 260 m above sea level, with an annual average temperature of 8.4°C and annual precipitation of 686 mm. The soil can be classified as *Gleyic* or *Eutric Cambisol*. Data has been taken at 50 points over the field site and at two reference sites (Fig. 8). Each measurement was for 900 seconds with the detector placed upon the ground and with measurements taken at the start of the study (15 measurements in a 6 hour period) and one week later (35 measurements over a 12 hour period). Core soil samples were collected from 3 locations and 1 reference site at the time of the second series of measurements.

Preliminary results show that radionuclides measured *in-situ* is a promising tool for improving the usefulness of the fallout radionuclides method to assess erosion at landscape level. Initial results showed changes in the measured caesium-137 level caused by geomorphological features beyond the uncertainties of our measurements. If verified, this technique would allow member states without traditional laboratory based gamma spectroscopy facilities to employ fallout radionuclides techniques in their efforts to improve soil conservation strategies at landscape level.

CAPACITY BUILDING AND ANALYTICAL SERVICES

Capacity building

Regional Training Course on Soil and Water Management in Agriculture to Support Crop Production in Asia and the Pacific

From 23 July to 17 August 2012, the SWMCN Subprogramme brought together twenty scientists and technicians from sixteen countries across the Asia and Pacific region (Afghanistan, Cambodia, China, Indonesia, Islamic Republic of Iran, Lao People's Democratic Republic, Malaysia, Myanmar, Nepal, Oman, Pakistan, Palau, Philippines, Sri Lanka, Vietnam and Yemen) for a training programme on soil and water management in agriculture. The emphasis of the training was on the use of isotopic and nuclear techniques to assess soil-water-crop processes and interactions, and to identify strategies to improve nutrient and water use efficiency and crop productivity. The training was funded by the Government of Japan under IAEA's Peaceful Uses Initiative (PUI) Programme.

Training was provided by the entire SWMCNL team in close collaboration with Mr Cepuder from the University of Natural Resources and Life Sciences, Vienna, Austria. As part of the training, the participants also attended the FAO/IAEA International Symposium on "Managing Soils for Food Security and Climate Change Adaptation and Mitigation", held at the IAEA in Vienna on 23 - 27 July 2012.

Besides the main objective of enhancing skills, knowledge and technical competence in soil, water, crop and nutrient management in agriculture, the training also provided an opportunity to share experiences, establish networks among the trainees and enhance cooperation between their countries.

The participants were privileged to also meet and interact with the Director General of the Food and Agricultural Organization (FAO) of the United Nations, Mr José Graziano da Silva, who was visiting the FAO/IAEA Agriculture and Biotechnology Laboratories (Fig. 9).

More information on this regional training course can be found on: *www.iaea.org/technicalcooperation/Regions/Asia-and-the-Pacific/News/05092012_Soil_Fellows.html*

FIG. 9. Trainees discuss food security issues with the Director-General of FAO during a training course on soil and water management in agriculture.

Individual Fellowship and Internship Training

In addition to the training course reported above, a group of fourteen fellows (six from Afghanistan, and one each from Bangladesh, Kenya, Mali, Oman, Sierra Leone, Sudan, United Republic of Tanzania and Zambia) received three months of intensive training in the application of isotopic and nuclear techniques to improve soil and water management and crop nutrition. Emphasis was placed on how to assess resource use efficiency. In addition, one fellow each from the Philippines and from Malaysia received individual training on the use of isotopic techniques to assess water and nutrient use in crops. Fellows learned how to conduct laboratory, glasshouse and field experiments for quantifying biological nitrogen fixation and agronomic water use efficiency and were also trained in the use of computer models, such as the AquaCrop model, to assess irrigation efficiency and to improve crop-water productivity. One Intern from Rumania received three months of training in the use of fallout radionuclide-based techniques for assessing soil erosion and redistribution, with a major focus on the use of beryllium-7.

Internet-Based Geospatial Information Visualization and Dissemination Platforms

The SWMCNL organized an internal training course from 3 to 14 December 2012 on the use of internet-based geospatial information visualization tools. The purpose of the training course was to provide basic knowledge and skills on how to disseminate and promote the work and research carried out by the Joint FAO/IAEA Division. The course focused on the use of internet-based geospatial information visualization and dissemination platforms (MapBox, Google Earth and others) that facilitate the storage, exchange and promotion of information in food and agriculture. The course described the various methods to provide and exchange information via the internet. By disseminating information via the internet, the Joint Division and its partners can reach target audiences more efficiently, which will assist in improving food and agricultural policy and programming in Member States. Such internet based tools can serve as invaluable tools in efforts to exchange and communicate information on soil conservation, water management and crop improvement across a wide audience.

Compound-Specific Stable Isotope Analysis of Fatty Acids to Identify Hot-Spots of Land Degradation and Developing Soil Conservation Strategies at Landscape Level

To promote internal capacity in the new area of Compound-Specific Stable Isotope Analysis, Christian Resch of the SWMCNL received a two-week training at the University of Hohenheim, Germany, in December 2012, in the use of Compound-Specific Stable Isotope Analysis of fatty acids to identify hot-spots of land degradation for developing cost-effective soil conservation strategies at landscape level. This training supports the SWMCN subprogramme on the importance of rapidly increasing land degradation in Member States, particularly in tropical and subtropical regions. With this expertise, the SWMCNL will now be able to initiate the dissemination of this novel analytical technique to Member States through group and individual fellowships.

Analytical services

A total of 4757 and 145 samples were analysed for stable isotopes and fallout radionuclides, respectively, at the SWMCNL. Most analyses were carried out to support research and development activities at the SWMCNL focused on the design of multiple robust and affordable isotope and nuclear techniques to improve soil and water management in climate-smart agriculture.

External Quality Assurance: Annual Proficiency Test on Nitrogen-15 and Carbon-13 Isotopic Abundance in Plant Materials

The annual Proficiency Test (PT) on nitrogen-15 and carbon-13 isotopic abundance in plant materials, jointly organized with the University of Wageningen, the Netherlands, and funded by the SWMCNL, was successfully completed. The Wageningen Evaluating Programs for Analytical Laboratories (WEPAL, *http://www.wepal.nl*) is accredited for the organization of Interlaboratory Studies by the Dutch Accreditation Council. Eleven stable isotope laboratories, as well as the SWMCNL, participated in the "IPE 2012.2" proficiency test (Argentina, Austria, Belgium, Brazil, Chile, Germany, Italy, Morocco, Pakistan, Turkey and Uruguay).

Participants received the WEPAL test sample set consisting of four samples of 20 g plant material each. All eleven laboratories reported isotope abundance data. Six out of eleven laboratories participating in the nitrogen analysis reported nitrogen-15 data within the control limits for the enriched plant sample and all eight participating laboratories in the carbon analysis reported carbon-13 isotopic abundance results within the control limits for this test sample.

Worldwide comparison of stable nitrogen-15 and carbon-13 isotope measurements will provide confidence in the participating laboratories' analytical performance and is hence an invaluable tool for external quality control. It is expected that in the future more stable isotope laboratories will make use of this unique opportunity to assess their analytical performance and hence be able to provide solid evidence of the quality of their analytical data.

Protocols and guidelines

Protocol development and dissemination of information via published journals and proceedings are major activities of the SWMCNL. Two protocols were prepared in 2012 and are expected to be released in 2013 on the use of fallout radionuclide and compound-specific stable isotope techniques for improving soil conservation strategies.

PUBLICATIONS

A total of 31 publications were generated in 2012. Twelve of these were published in international peer-reviewed journals. A further fifteen were presented during the FAO/IAEA International Symposium on "Managing Soils for Food Security and Climate Change Adaptation and Mitigation", 23-27 July 2012, Vienna, Austria.

Adu-Gyamfi, J., Aigner, M., Gludovacz, D., Linic, S. (2012). Phosphorus acquisition from sparingly soluble forms by maize and soybean in low- and medium-P soils using ^{32}P. Book of Abstracts, International Symposium on "Managing Soils for Food Security and Climate Change Adaptation and Mitigation", 23-27 July 2012, Vienna, Austria, pp. 101-102.

Adu-Gyamfi, J., Arrillaga, J., Linic, S., Heng, L. (2012). Oxygen isotope composition and carbon isotope discrimination for evaluating wheat lines for tolerance to pre- and post-anthesis water stress. Book of Abstracts, International Symposium on "Managing Soils for Food Security and Climate Change Adaptation and Mitigation", 23-27 July 2012, Vienna, Austria, pp. 317-318.

Adu-Gyamfi, J., Dercon, G. (2012). Overview of FAO/IAEA Coordinated Research Project on crop genotypes tolerant to low N and P soils. Book of Abstracts, International Symposium on "Managing Soils for Food Security and Climate Change Adaptation and Mitigation", 23-27 July 2012, Vienna, Austria, pp. 29-30.

Asfary, A.F., Wahbi, A., Hazzouri, A., Sattouf, M., Rahal, K., Makhlof, M., Toloza, A., Mabit, L. (2012). Assessment of soil erosion rates under degraded forest, olive groves and wheat/fallow in semi-arid regions of Syria. Book of Abstracts, International Symposium

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Aulakh, M., Manchanda, J.S., Garg, A., Kumar, S., Dercon, G., Nguyen, M.L. (2012). Crop production and nutrient use efficiency of conservation agriculture for soybean-wheat rotation in the Indo-Gangetic Plains of Northwestern India. Soil and Tillage Research, 120:50-60.

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Dercon, G., Heiling, M., Aigner, M., Mayr, L., Resch, C., Bodé, S., Boeckx, P., Arrillaga, J., Cepuder, P., Bodner, G., Nolz, R., Adu-Gyamfi, J., Nguyen, M.L. (2012). Assessment of carbon distribution and soil organic carbon storage in mulch-based cropping systems by using isotopic techniques. International Symposium on "Managing Soils for Food Security and Climate Change Adaptation and Mitigation", 23-27 July 2012, Vienna, Austria, pp. 247-248.

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EXTERNAL COLLABORATIONS AND PARTNERSHIPS

Institution	Topic
Austrian Agency for Health and Food Safety, Austria	Assessment of carbon distribution and soil organic carbon storage in mulch-based cropping systems by using isotopic techniques Assessing soil erosion at landscape level by means of the fallout radionuclides method via in-situ gamma spectrometry
Centre national de l'énergie, des sciences et des techniques nucléaires (CNESTEN), Morocco	Development of protocol for the use of fallout radionuclide techniques for improving soil conservation strategies
CSIRO Land and Water, Canberra, Australia	Partitioning of soil evaporation using conventional and isotopic techniques
Eidgenössische Technische Hochschule (ETH), Switzerland	Assessment of carbon distribution and soil organic carbon storage in mulch-based cropping systems by using isotopic techniques Use of oxygen-18 isotopes in phosphate to trace phosphorus sources and cycling in soils
Fujian Agriculture and Forestry University, China	Use of oxygen-18 isotopes in phosphate to trace phosphorus sources and cycling in soils
National Institute of Water and Atmospheric Research, New Zealand	Development of protocol for the use of compound-specific stable isotope techniques for improving soil conservation strategies
Universidad Austral de Chile, Chile	Development of protocol for the use of fallout radionuclide techniques for improving soil conservation strategies
University of Basel, Switzerland	Development of protocol for the use of compound-specific stable isotope techniques for improving soil conservation strategies
University of Birmingham. UK	Assessing soil erosion at landscape level by means of the fallout radionuclides method via *in-situ* gamma-ray spectrometry
University of Exeter, UK	Development of protocol for the use of compound-specific stable isotope techniques for improving soil conservation strategies

Institution	Topic
University of Ghent, Belgium	Assessment of carbon distribution and soil organic carbon storage in mulch-based cropping systems by using isotopic techniques Development of protocol for the use of compound-specific stable isotope techniques for improving soil conservation strategies
University of Hohenheim, Germany	Training in the use of compound-specific stable isotope techniques for improving soil conservation strategies Development of protocol for the use of compound-specific stable isotope techniques for improving soil conservation strategies
University of Leuven, Belgium	Assessment of carbon distribution and soil organic carbon storage in mulch-based cropping systems by using isotopic techniques
University of Natural Resources and Life Sciences, Vienna, Austria	Validating the components of evapotranspiration from soil evaporation and plant transpiration, with oxygen-18 isotopes and micrometeorology Assessment of carbon distribution and soil organic carbon storage in mulch-based cropping systems by using isotopic techniques
University of Plymouth, UK	Beryllium-7 as short-term radiotracer of soil movement: potential and limitations Development of protocol for the use of fallout radionuclide techniques for improving soil conservation strategies
University of Vienna, Austria	Assessment of carbon distribution and soil organic carbon storage in mulch-based cropping systems by using isotopic techniques

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